UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

JOHN HANCOCK LIFE INSURANCE COMPANY, JOHN HANCOCK VARIABLE LIFE INSURANCE COMPANY, and MANULIFE INSURANCE COMPANY (f/k/a INVESTORS PARTNER LIFE INSURANCE COMPANY),	)	Civil Action No. 05-11150-DPW
Plaintiffs,	)	
v.	)	
ABBOTT LABORATORIES,	)	
Defendant.	)	

ABBOTT'S MOTION FOR LEAVE TO FILE A REPLY IN SUPPORT OF ITS MOTION TO OVERRULE HANCOCK'S OBJECTIONS TO ABBOTT TRIAL EXHIBITS

Pursuant to Local Rule 7.1(B)(3), Defendant Abbott Laboratories ("Abbott') requests leave to file the attached [Proposed] Reply in Support of Its Motion to Overrule Objections to Abbott Trial Exhibits ("Reply"). *See* Ex. A. Abbott's proposed reply is brief (slightly over three pages, plus one exhibit) and responds only to new arguments Hancock raises for the first time in its opposition to the motion.

For the above reasons, Abbott respectfully requests leave to file the attached Reply Memorandum.

Dated: May 2, 2008

Respectfully submitted,

ABBOTT LABORATORIES

By: _____/s/ Eric J. Lorenzini_____

One of its attorneys

Peter E. Gelhaar (BBO#188310)
Michael S. D'Orsi (BBO #566960)
DONNELLY, CONROY & GELHAAR LLP
1 Beacon St., 33rd Floor
Boston, Massachusetts 02108
(617) 720-2880
peg@dcglaw.com
msd@dcglaw.com

and

Jeffrey I. Weinberger (*pro hac vice*)
Gregory D. Phillips (*pro hac vice*)
Eric J. Lorenzini *(pro hac vice)*
Ozge Guzelsu *(pro hac vice)*
MUNGER, TOLLES & OLSON LLP
355 South Grand Avenue
Thirty-Fifth Floor
Los Angeles, CA 90071-1560
Tele: (213) 683-9100

Counsel for Abbott Laboratories

LOCAL RULE 7.1 CERTIFICATION

The undersigned hereby certifies that Abbott's counsel and Hancock's counsel conferred regarding this motion but the parties were unable to reach agreement.

/s/ Eric J. Lorenzini

CERTIFICATE OF SERVICE

I hereby certify that this document(s) filed through the ECF system will be sent electronically to the registered participants as identified on the Notice of Electronic Filing (NEF) and paper copies will be sent to those indicated as non registered participants on May 2, 2008.

Date: May 2, 2008.

/s/ Eric J. Lorenzini

Exhibit 1

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

JOHN HANCOCK LIFE INSURANCE COMPANY, JOHN HANCOCK VARIABLE LIFE INSURANCE COMPANY, and MANULIFE INSURANCE COMPANY (f/k/a INVESTORS PARTNER INSURANCE COMPANY), Plaintiffs, v. ABBOTT LABORATORIES, Defendants.	CIVIL ACTION NO. 05-11150-DPW Hon. Judge Douglas P. Woodlock

ABBOTT'S [PROPOSED] REPLY IN SUPPORT OF ABBOTT'S MOTION TO OVERRULE HANCOCK'S OBJECTIONS TO ABBOTT TRIAL EXHIBITS

Defendant Abbott Laboratories ("Abbott") respectfully submits this reply in support of its motion to overrule Hancock's objections to Abbott trial exhibits, filed on April 4, 2008.

ARGUMENT

Abbott's opening brief explains in detail why Hancock's objections should be overruled. For the sake of brevity, Abbott will not repeat those arguments here, and will instead respond to new arguments Hancock raises for the first time in its opposition to the motion.

First, in response to Abbott's offer of certain emails that provide context for attached documents (to which Hancock does not object), Hancock asserts that "the fact that an Abbott employee sent or received these communications is irrelevant to any of the issues in dispute." Hancock's Opp. to Abbott's Mot. to Overrule and Cross Mot., filed April 18, 2008 ("Hancock Opp") at 2; Exs. [946], [973], [980]. Hancock's assertion is incorrect. The emails provide

information regarding the authors, recipients, and date of distribution of the attached documents, all of which information is helpful to a fully informed evaluation of those documents.[1]

Second, Hancock asserts that five memoranda and one email do not fall within the business records exception because "there must be some evidence of a business duty to make and regularly maintain records of this type." Hancock Opp. at 2; Exs. [959], [967], [968], [969], [970], and [972]. However, the evidence supports that documents such as Monthly Highlights memoranda are regularly maintained pursuant to a business duty. *See, e.g.*, Leonard Aff. ¶ 54 (Dr. Leonard prepared Monthly Highlights Memoranda).

Third, with respect to documents offered to show state of mind or that information was publicly available, Hancock argues that "Abbott fails to establish any foundation that any of the Proposed Exhibits actually were provided to, or reviewed by, anyone at John Hancock." Hancock Opp. at 3. Documents generally available at the time of the agreement are relevant, however, because a party seeking rescission must show actual *and reasonable* reliance on the alleged misrepresentation. *See* Ex. [947], [949], [950]; *Pension Benefit Guar. Corp. v. Ziffer*, No. 91C7762, 1994 U.S. Dist. LEXIS 87, *28 (N.D. Ill. Jan. 4, 1994); *see also* Ex. 32 (RFA) § 12.2(i) (agreement excludes generally available information regarding the pharmaceutical industry in general from the representations and warranties clause). Exhibit [993], a recent press

[1] Hancock also incorrectly asserts that there is no basis for concluding that the email and the attached "ABT-518 DDC document" offered as Exhibit [973] "actually are related." Hancock Opp. at 2 n.1. It is clear from the face of the email that the "DDC document" is attached to the email. The email expressly references attachment of a Word copy of the "ABT-518 DDC document", as well as a PDF copy of a Journal of Clinical Oncology article regarding the Bayer MMPI compound. Ex. [973] at p. 1. The Bates numbers of the email and the DDC document are non-sequential only because Abbott omitted the irrelevant journal article. To avoid any doubt that the documents are related, Abbott attaches hereto as Exhibit A is a true and correct copy of the email with both attachments. As noted in Abbott's opening brief, Hancock does not object to admission of the DDC document, only the email. Abbott respectfully urges admission of the email, as well as the DDC document, to provide context (e.g., to show when the DDC document was distributed). Alternatively, Abbott requests admission of the email with both attachments or admission of the DDC document as a stand-alone exhibit.

release regarding Advanced Life Science's development of ABT-773, is offered as an example of available information that Hancock and its damages expert failed to consider in their damages assessment. Exhibit [993] is thus relevant to the credibility and reliability of that assessment. Hancock argues that documents offered to show Abbott's state of mind are inadmissible because of purported lack of evidence regarding "whether and when the exhibit was reviewed by decision makers within Abbott." Hancock Opp., Ex. A. However, all but one of the exhibits are internal Abbott documents, *see* Ex. [952], [953], [974], and the remaining document was published in a medical journal, *see* [997], thus they are relevant to Abbott's knowledge and state of mind. Any lack of specificity regarding whether the documents were directly reviewed and relied upon by "decision-makers" goes to the weight of the evidence, not admissibility.

Fourth, Hancock incorrectly argues that drafts of the Research Funding Agreement ("RFA") are irrelevant because "Abbott consistently has maintained that the 'plain language' of § 3.3(b) is unambiguous by its terms." Hancock Opp. at 4. Abbott has argued that the plain language of the RFA, when read as a whole and taking into account the rule against penalty clauses, does not support Hancock's Section 3.3(b) claim. However, Abbott has also consistently argued, in the alternative, that parol evidence demonstrates that the parties did not intend Section 3.3(b) to apply in the current circumstances. *See, e.g.*, Abbott's Reply in Supp. of Mot. to Dismiss & Opp. to Hancock's Mot. for Part. Summ. Judg., filed Oct. 16, 2006 (Dkt. No. 57) at 8-9 n.3; Abbott's Supp. Mem. in Opp. to Hancock's Cross-Mot. for Part. Summ. Judg., Nov. 22, 2006 (Dkt. No. 83); Abbott's Supp. Mem. in Supp. of Mot. to Dismiss & Opp. to Hancock's Cross-Mot. for Part. Summ. Judg., filed Dec. 13, 2006 (Dkt. No. 99), at 1-2, 11-12.[2]

Finally, Hancock withdraws its objections to Exhibits [948], [962], [982], [984], and

[2] Hancock's arguments concerning Exhibits [964], [965], and [960] are addressed in Abbott's opening brief and will not be repeated here. *See* Abbott's Mot. at 4-5, 11.

[992] and concedes that the Court may take judicial notice of the Court transcripts marked as Exhibits [966] and [971]. Hancock Opp. at Ex. A.

I. CONCLUSION

For the reasons stated above, Abbott respectfully requests that the Court overrule Hancock's objections to the exhibits offered by Abbott.

Dated: May 2, 2008

ABBOTT LABORATORIES

By its attorneys

/s/ Eric J. Lorenzini

Michael S. D'Orsi
Peter E. Gelhaar (BBO #188310)
Michael S. D'Orsi (BBO #566960)
DONNELLY, CONROY & GELHAAR LLP
1 Beacon St., 33rd Floor
Boston, Massachusetts 02108
(617) 720-2880

and

Jeffrey I. Weinberger (Admitted Pro Hac Vice)
Gregory D. Phillips (Admitted Pro Hac Vice)
Eric J. Lorenzini (Admitted Pro Hac Vice)
Ozge Guzelsu (Admitted Pro Hac Vice)
MUNGER, TOLLES & OLSON LLP
355 South Grand Avenue, 35th Floor
Los Angeles, CA 90071
(213) 683-9100

Counsel for Abbott Laboratories

CERTIFICATE OF SERVICE

I hereby certify that this document(s) filed through the ECF system will be sent electronically to the registered participants as identified on the Notice of Electronic Filing (NEF) and paper copies will be sent to those indicated as non registered participants on May 2, 2008.

Date: May 2, 2008.

/s/ Eric J. Lorenzini

Exhibit A
Part 1

Steven K Davidsen/LAKE/PPRD/ABBOTT
1110312000 06:14 AM

To: Todd J Janus/LAKE/PPRD/ABBOTT@ABBOTT, Diane L D'Amico/LAKE/PPRD/ABBOTT@ABBOTT
cc:
bcc:
Subject: Re: JCO paper on Bayer Compound

Todd & Diane,

Thanks for the reference. One review that I found particularly useful was written by Kevin Lynch from Novartis (Inter. J. Pharm. Med. 1999, 13, 127-136) - he does an excellent job of describing the issues associated with the development of MMP inhibitors

Presumably you and Diane have electronic versions of the ABT-518 DDC document,..? I've enclosed a copy if not. I also placed a hardcopy in the housemail for Diane.

With respect to cytotoxicity measurements, please replace "MTT" with "Alamar Blue" in the second sentence on page 21.... cell culture aficionados are more familiar with the MTT assay, however for this document the slight distinction doesn't matter.

Steve

Backup MMP DDC Write-up Final
Todd J Janus

Todd J Janus
11/02/2000 04:13 PM

To: Steven K Davidsen/LAKE/PPRD/ABBOTT@ABBOTT
Subject: JCO paper on Bayer Compound

Steve,
Here is the reference on the Baycr Compound and the PDF file
Also - can I get another copy of the DDC doc for Diane D'Amico?
thanks

JCO 18-178 pdf

Eric K. Rowimky, Rachel Humphrey, Lisa A Hammond, Cheryl Aylesworth, Leslie Smetzer, Manuel Hidalgo, Mark Morrow, Lon Smith, Allison Garner, J Mel Sorensen, Daniel D. Von Hoff, and S. Gail Eckhardt
Phase I and Pharmacologic Study of the Specific Matrix Metalloproteinase Inhibitor BAY 12-9566 on a Protracted Oral Daily Dosing Schedule in

Patients With Solid Malignancies
J Clin Oncol 2000 18: 178

Phase I and Pharmacologic Study of the Specific Matrix Metalloproteinase Inhibitor BAY 12-9566 on a Protracted Oral Daily Dosing Schedule in Patients With Solid Malignancies

By Eric K. Rowinsky, Rachel Humphrey, Lisa A. Hammond, Cheryl Aylesworth, Leslie Smetzer, Manuel Hidalgo, Mark Morrow, Lon Smith, Allison Garner, J. Mel Sorensen, Daniel D. Von Hoff, and S. Gail Eckhardt

Purpose: **To evaluate the feasibility of administering BAY 12-9566, a matrix metalloproteinase (MMP) inhibitor with relative specificity against MMP-2, MMP-3, and MMP-9, on a protracted oral daily dosing schedule in patients with advanced solid malignancies. The study also sought to determine the principal toxicities of BAY 12-9566, whether plasma BAY 12-9566 steady state concentrations (C_{ss}) of biologic relevance could be sustained for prolonged periods, and whether BAY 12-9566 affected plasma concentrations of MMP-2, MMP-9, and tissue inhibitor of MMP-2 (TIMP-2).**

Patients and Methods: **Patients with solid malignancies were treated with BAY 12-9566 at daily oral doses ranging from 100 to 1,600 mg. BAY 12-9566 dose schedules included 100 mg once daily, 400 mg once daily, 400 mg twice daily, 400 mg three times daily, 400 mg four times daily, and 800 mg twice daily. Plasma was collected to study the range of BAY 12-9566 C_{ss} values achieved, and exploratory studies were performed to assess the effects of BAY 12-9566 on plasma concentrations of MMP-2, MMP-9, and TIMP-2.**

Results: **Twenty-one patients were treated with 47 28-day courses of BAY 12-9566. The most common side effects were headache, nausea, vomiting, abnormalities in hepatic functions, and thrombocytopenia, which were rarely clinically significant. BAY 12-9566 was well tolerated on all dose schedules, and there was no consistent dose-limiting toxicity that precluded treatment in the range of dose schedules evaluated. Instead, dose escalation was terminated because BAY 12-9566 plasma C_{ss} values increased less than proportionately and plateaued as the daily dose was increased within the dose range of 100 to 1,600 mg/d, suggesting saturable drug absorption. Mean plasma C_{ss} values achieved with all dose schedules exceeded BAY 12-9566 concentrations required to inhibit MMPs in vitro and in vascular invasion and tumor proliferation in vivo models. There were no consistent effects of BAY 12-9566 on the plasma concentrations of MMP-2 and MMP-9 over the continuous dosing period at any dose schedule level. However, plasma levels of TIMP-2 seemed to increase in a dose-dependent manner ($r^2 = .50$, $P = .046$).**

Conclusions: **The recommended dose of BAY 12-9566 for subsequent disease directed studies is 800 mg twice daily, which resulted in biologically relevant plasma C_{ss} values and an acceptable toxicity profile. Although exploratory studies of MMPs in plasma were not revealing, it is conceivable that some tumor types and disease settings are more likely to produce more readily quantifiable levels of activated MMPs than others. Therefore, attempts to identify and quantify surrogate markers of MMP inhibitory effects should continue to be performed in disease-directed studies in more homogenous patient populations.**

J Clin Oncol 18:178-186.

MATRIX Metalloproteinases (MMPs) are a family of secreted or transmembrane proteins that are capable of digesting the extracellular matrix and basement membrane components under physiologic conditions.[1,2] The three major subgroups of MMPs, classified by their substrate preferences, include collagenases, which degrade collagen; stromelysis, which prefer proteoglycans and glycoproteins; and gelatinases, which are especially potent at digesting nonfibrillar and denatured collagen (gelatin).[2-5] The MMPs are secreted as latent proenzymes and require activation by a series of proteolytic enzymes. Once activated, they may be inhibited by the general serum proteinase inhibitor α2-macroglobulin and a family of specific tissue inhibitors called tissue inhibitors of MMPs (TIMPs).[2-7] MMPs and TIMPs are usually secreted by stromal cells and macrophages of normal tissues, as well as by vascular endothelial cells. Malignant neoplasms are capable of synthesizing and activating MMPs, particularly MMP-2 and MMP-9, and studies in which MMPs or TIMPs have been manipulated genetically or pharmacologically suggest that both factors are key regulators of tumor growth at both primary and metastatic sites.[1-13]

From the Institute for Drug Development, Cancer Therapy and Research Center; University of Texas Health Science Center; and Brooke Army Medical Center, San Antonio, TX; and Pharmaceuticals Division, Bayer Corporation, West Haven, CT.

Submitted May 13, 1999; accepted August 31, 1999.

Supported by a research grant from Bayer Corporation, Pharmaceuticals Division, West Haven, CT.

Presented in part at the Thirty-Fourth Annual Meeting of the American Society of Clinical Oncology, Los Angeles, CA, May 16-19, 1998.

Address reprint requests to Eric Rowinsky, MD, Institute for Drug Development, Cancer Therapy and Research Center, 8122 Datapoint Dr, Ste 700, San Antonio, TX 78229; email erowinsk@saci.org.

0732-183X/00/1801-178

The net degradative effects of the MMPs on the extracellular matrix reflect a dynamic equilibrium between the secretion and activation of MMPs and TIMPs.[1-13] The impact of this dynamic equilibrium and the various effects of specific MMPs and TIMPs on the invasiveness and metastatic potential of malignant neoplasms are complex and not fully understood.[14-16] However, the overall actions of the MMPs in degrading the extracellular matrix and the contribution of these effects on the aggressiveness of malignant tumors seem to be more closely related to the net expression of activated MMPs and TIMPs than the absolute levels of any specific MMP or TIMP.[1-3,5,17-19] The complexity of the interactions between MMPs and TIMPs on the various intracellular and extracellular compartments of both tumors and stromal tissues also confounds efforts directed at identifying components of these processes that accurately reflect the net effects of MMPs and TIMPs in malignant tumors and that can be measured reliably in the clinic.[20,21]

Based on the results of preclinical studies delineating the critical roles of MMPs in tumor invasion, malignant angiogenesis, and metastases, therapeutic strategies targeting MMPs and subcellular constituents involved in the degradation of the basement membrane and extracellular matrix are being evaluated in patients with malignant diseases.[3,4,22,23] BAY 12-9566 (Fig 1) is an orally bioavailable biphenyl compound that was originally synthesized and screened for inhibitory activity against MMP-3 (stromelysis). In addition to inhibiting MMP-3, BAY 12-9566 is a potent inhibitor of the critical enzymes MMP-2 and MMP-9, but it is much less effective at inhibiting MMP-1, which seems to be associated with the development of polyarthritis and tendonitis.[20,22-24] In preclinical studies, the inhibitory activities of BAY 12-9566 in both in vitro and in vivo models of matrix invasion, malignant angiogenesis, and tumor growth were notable.[24-31]

BAY 12-9566 was active in various models of tumor metastases.[25,26] In a subcutaneously implanted Lewis lung carcinoma that spontaneously metastasized to the lungs, daily oral treatment of mice with BAY 12-9566 beginning 3 days after implantation delayed the growth of primary tumors by 50% and reduced the total number of lung metastases and lung lesions greater than 3 mm^3 by 86% and 90%, respectively.[25,26] In an experimental metastasis model in which B16.F10 murine melanoma cells were injected into the tail vein of mice, treatment with BAY 12-9566 from 24 hours before implantation to 48 hours after implantation reduced the total number of lung metastases and lesions greater than 2 mm^3 by 58% and 80%, respectively.[25,26] BAY 12-9566 also inhibited the growth of well-established subcutaneous xenografts of human colon carcinomas.[28,29] Furthermore, the agent inhibited growth and metastases of orthotopically-implanted human HCT 16 colon cancer, which more closely resembles the clinical situation with regard to tumor-stromal interactions than tumor xenografts. Therefore, BAY 12-9566 may be superior to traditional xenografts to screen for compounds that inhibit matrix invasion, malignant angiogenesis, and metastasis.[28,29]

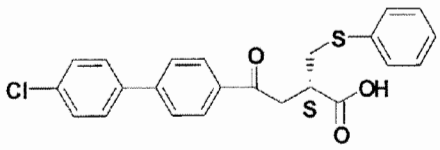


Fig 1. Structure of BAY 12-9566.

Preclinical pharmacologic studies in mice, rats, guinea pigs, and dogs indicated that BAY 12-9566 is highly bioavailable (70% to 98%) after oral administration, with peak plasma concentrations attained by 0.5 to 2 hours.[32] In rats and dogs, drug disposition was almost exclusively through hepatic metabolism and biliary excretion of both parent compound and metabolites.[32] Hepatotoxicity, characterized by reversible elevations in serum transaminases, was the principal toxic effect of BAY 12-9566 in both rodents and dogs.[30] BAY 12-9566 also modestly depressed erythropoiesis and produced a reversible tubular nephropathy in female rats.[30] In vitro studies of the effects of BAY 12-9566 on P450 subfamilies have also indicated that the agent would only be a weak inducer of human P450 isoenzymes.[30] In healthy human volunteers treated with BAY 12-9566 at doses up to 100 mg/d, the pharmacokinetics were linear; however, higher doses resulted in less than proportionate increases in plasma drug concentrations.[31]

The principal objectives of this study were to: (1) characterize and quantify the toxicities of BAY 12-9566 administered on a protracted daily oral dosing schedule in patients with advanced solid malignancies; (2) determine the maximum-tolerated dose of BAY 12-9566 on a oral protracted daily dosing schedule; (3) evaluate the range of steady state BAY 12-9566 plasma concentrations (C_{ss}) that can be sustained for protracted durations; (4) seek preliminary information about the effects of BAY 12-9566 on plasma concentrations of specific MMPs and TIMPs that are of potential clinical relevance; and (5) seek preliminary evidence of antitumor activity and clinical benefit.

PATIENTS AND METHODS

Eligibility

Patients with histologically or cytologically documented assessable or measurable solid malignancies refractory to conventional chemo-

therapy or for whom no effective therapy existed were candidates for this study. Eligibility criteria also included: (a) age ≥ 18 years; (b) an Eastern Cooperative Oncology Group performance status ≤ 2 (capable of self-care); (c) a life-expectancy of at least 12 weeks; (d) no treatment with investigational or cytotoxic agents within 28 days, mitomycin C and nitrosoureas within 42 days, or wide-field radiation within 14 days of entering onto study; (e) adequate hematopoietic function (absolute neutrophil count ≥ 1,500/μL and platelet count ≥ 100,000/μL), hepatic function (serum bilirubin < 1.5 mg/dL; AST and ALT ≤ two times the upper limit of institutional normal values; and alkaline phosphatase ≤ four times the upper limit of the institutional normal value), and renal function (serum creatinine < 2.0 mg/dL); (f) no history of a major cardiovascular event within 3 months of entering onto study; (g) no known history of hepatitis or human immunodeficiency viral infection; (h) no history of hypersensitivity to any of the components of the study medications, significant drug allergies, or any clinically significant hypersensitivity disorder; (i) no history of primary or metastatic malignant involvement of the CNS; (j) no history or evidence of gastrointestinal disease that could potentially interfere with the absorption of the study medication; and (k) no coexisting medical or psychiatric problems of sufficient severity to limit full compliance with the study. All patients gave written informed consent before treatment according to federal and institutional guidelines.

Study Design, Dosage, and Drug Administration

The starting dose of BAY 12-9566 was 100 mg/d orally, which was previously demonstrated to be a safe dose in healthy volunteers.[31] Patients were treated at the following dose levels: 100 mg once daily, 400 mg once daily, 400 mg twice daily (every 12 hours), 400 mg three times daily (every 8 hours) daily, 400 mg four times daily (every 6 hours), and 800 mg twice daily (every 12 hours). Tablets were swallowed intact followed by the ingestion of one glass of water. Treatment was continuous, and each 28-day period constituted a single course of therapy. Because food affected neither the absorption nor pharmacokinetics of BAY 12-9566 in a prior healthy volunteer study, there were no specific meal requirements in the before and during the treatment period.[31] Similarly, although concurrent medications were recorded and every attempt was made to discontinue medications known to generally affect hepatic metabolism and gastrointestinal absorption, there were no specific restrictions regarding the coadministration of other medications during any phase of the study.

At least three new patients were to be treated at each dose level. Dose escalation in new patients was permitted if no dose-limiting toxicity (DLT) was experienced by a minimum of three patients treated for at least 28 days. If one of three patients experienced DLT during the first 28-day period, at least three additional patients were treated. Intrapatient dose escalation was not permitted. Dose reduction by one dose level was permitted for patients who experienced DLT, which was defined as (1) any grade 3 or 4 toxicity; (2) symptomatic grade 2 toxicity on optimal pharmacologic management; and (3) any grade 2 biochemical toxicity that persisted longer than 7 days. The dose of BAY 12-9566 was held for patients who developed more than or equal to grade 3 toxicity or grade 2 toxicity associated with symptoms or biochemical abnormalities lasting longer than 7 days. After resolution of the toxicity to less than or equal to grade 1, treatment with BAY 12-9566 was resumed at either the same or a reduced dose. If recovery to grade 1 toxicity did not occur within 3 weeks, the patient was withdrawn from the study. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria. The recommended dose for subsequent phase II studies was defined as the highest dose level in which less than two of six new patients experienced DLT.

BAY 12-9566 was supplied by Bayer Corporation, Pharmaceutical Division (West Haven, CT) as 12.5-mg, 50-mg, or 200-mg tablets containing micronized BAY 12-9566, anhydrous lactose, microcrystalline cellulose, sodium lauryl sulfate, croscarmelloe sodium, magnesium stearate, and purified water, which was removed during the granulation drying procedure.

Pretreatment and Follow-Up Studies

Histories, physical examinations, and routine laboratory studies were performed within 7 days before treatment with BAY 12-9566. Routine laboratory studies consisted of a complete blood count, electrolytes, chemistries, clotting times, and urinalysis. These routine studies were also repeated weekly. Histories and physical examinations were repeated weekly during the first course of treatment and immediately before each course thereafter. An ECG was performed before the first course. Sites of malignant disease were formally measured before treatment and after every two courses using physical examination and standard radiographic procedures. Treatment was continued in the absence of progressive disease or unacceptable toxicity. A complete response was defined as disappearance of all disease on two measurements separated by a minimum of 4 weeks, whereas a partial response required a greater than 50% decrease in the sum of the product of the bidimensional measurements of all measurable lesions documented by two measurements separated by at least 4 weeks.

Pharmacologic Studies of BAY 12-9566

The principal goal of the pharmacologic studies was to evaluate the range of plasma BAY 12-9566 C_{ss} achieved. Venous blood samples (7 mL) were placed into tubes containing sodium heparinate. In the first three patients, samples were collected before treatment and 1, 2, 4, and 8 hours after treatment on day 1, 24 hours after treatment (immediately before treatment on day 2), and immediately before treatment on days 8, 15, 29, and 57. In all other patients, blood was collected before treatment on day 1 and immediately before treatment on days 8, 15, 29, and 57. The samples were immediately centrifuged at 2,800 revolutions per minute for 10 minutes, and the plasma was stored at −70°C.

The procedures used for sample extraction and high-performance liquid chromatography (HPLC) were previously reported.[33] Briefly, 0.5 mL of plasma was added to 1 mL of a 0.5% phosphoric acid in acetonitrile solution containing BAY 13-8825 internal standard (4-[4-{chlorophenyl}phenyl]-4-oxo-2*S*-[4-ethylphenylthiomethyl]) butanoic acid. After centrifugation, a 50- to 75-μL sample was injected onto the HPLC system. An Ultrasphere C8 5 mm, 250 × 4.6–mm column (Beckman, Palo Alto, CA) was used for a gradient elution followed by reverse-phase HPLC separation using ultraviolet detection at 280 nanometers (nm). The mobile phase consisted of varying proportions of solution A (100 mmol/L acetate buffer, pH 3.5) with solution B (100 mmol/L acetate buffer, pH 3.5 solution/acetonitrile [10/90 volume per volume]) at a flow rate ranging from 0.8 to 1.8 mL/min. Retention times were 16.2 to 16.7 minutes and 19.8 to 20.6 minutes for BAY 12-9566 and BAY 13-8825, respectively. Standard curves were formulated using known BAY 12-9566 concentrations (0.05 to 50 μg/mL). BAY 12-9566 plasma concentrations were derived by linear correlation based on known concentrations of BAY 13-8825 and the relative peak height response ratio of BAY 12-9566 to the internal standard. The standard linear regression curves of concentration versus peak height response ratios were first order with a 1/yr weighting factor. The assay had a lower limit of detection of 0.02 μg/mL, a lower limit of quantification of 0.05 μg/mL, a precision ranging from 0% to 6.23% relative standard deviation, and an accuracy exceeding 91.3%.

MMP and TIMP-2 Studies

Seven-mL blood samples were collected into venipuncture tubes that contained ethylenediaminetetraacetic acid before treatment on day 1, immediately before treatment weekly for 4 weeks, and then every 2 weeks, thereafter. The samples were immediately centrifuged at 2,800 revolutions per minute for 10 minutes, and plasma was stored at −30°C. Quantification of MMP-2 (pro-MMP-2 and MMP-2 complexed to TIMP-2) and MMP-9 (pro-MMP-9 and MMP-9 complexed to TIMP-1) concentrations in plasma was performed using "sandwich" ELISA kit formats (Biotrak; Amersham Pharmacia, Piscataway, NJ) in which 96-well plates were precoated with primary polyclonal antibodies to MMP-2 or MMP-9 and detection was accomplished using a secondary antibody to horseradish peroxidase. A similar system was used to measure TIMP-2 concentrations in plasma; however, the primary polyclonal antibody to TIMP-2 recognizes free TIMP-2 plus TIMP-2 complexed to active MMPs but not TIMP-2 complexed to pro-MMP. Briefly, diluted plasma samples (1/100 for MMP-2; 1/10 for MMP-9; 1/4 for TIMP-2) were incubated with the primary antibody for 1 to 2 hours at room temperature, followed by washing and incubation with the secondary antibody for 1 to 2 hours. After washing and removing the antibody, spectrophotometric detection was accomplished using 100 μL of tetramethylbenzidine for color development and measurement at 450 nm with a microtiter plate spectrophotometer. The lower limits of sensitivity of the assays for MMP-2, MMP-9, and TIMP-2, which were defined as two SDs above the mean optical density of 30 standard zero replicates, were 0.37, 0.6, and 0.3 ng/mL, respectively.

Active MMP-9 was measured using a similar Biotrak "sandwich" ELISA kit. Ninety-six well plates were precoated with polyclonal antibodies to MMP-9 to bind both free active and pro-MMP-9. To measure the bound active MMP-9 fraction after incubation with the primary antibody, the sample was incubated with a pro-form of a detection enzyme (*S*-2444) that is cleaved in the presence of active MMP-9 to yield a chromogenic peptide product. Plasma was diluted 1/32 with a buffer solution. Standards and samples were initially incubated in microtiter wells precoated with antiMMP-9 antibody. After an overnight incubation at 4°C followed by washing, 50 μL of detection solution, which consisted of the *S*-2444 peptide substrate and a detection enzyme solution composed of modified urokinase in 50 mmol/L Tris HCl buffer pH 7.6, 150 mmol/L sodium chloride, 5 mmol/L calcium chloride, 1 μmol/L zinc chloride, and 0.01% BRIJ 35 (Biotrak), was added to each well. The resultant color was measured at 405 nm using a microtiter plate spectrophotometer. The lower limit of assay sensitivity was 500 pg/mL.

RESULTS

General

Twenty-one patients, whose characteristics are listed in Table 1, were treated with 47 28-day courses of BAY 12-9566 through six discrete dose schedule levels, as listed in Table 2. Prior treatment consisted of at least chemotherapy in 20 subjects and immunotherapy only in one subject. The median number of therapy courses administered was two (range, one to six courses). Two individuals required dose schedule modifications for toxicity. In one of these subjects, the BAY 12-9566 dose schedule was modified from 400 mg three times daily to 400 mg twice daily because of an isolated grade 3 elevation in serum bilirubin. The other individual experienced protracted grade 2 nausea, vomiting, and malaise, resulting in a BAY 12-9566 dose schedule modification from 400 mg four times daily to 400 mg three times daily.

A maximum-tolerated BAY 12-9566 dose was not determined in the range of total daily doses evaluated, and the feasibility of administering higher total daily doses was not assessed because plasma BAY 12-9566 C_{ss} values exceeded

Table 1. Patient Characteristics

Characteristic	No. of Patients
Total patients assessable	21
No. of courses per patient	
Median	2
Range	1-6
Age, years	
Median	54
Range	33-75
Sex	
Male	10
Female	11
Median performance status (ECOG)	1
0	8
1	11
2	2
Previous therapy	
Chemotherapy + radiation ± immunotherapy	10
Chemotherapy + immunotherapy and/or hormonal therapy	6
Chemotherapy only	4
Immunotherapy only	1
Tumor types	
Colorectal	4
Kidney	4
Sarcoma	3
Melanoma	2
Breast	2
Unknown primary, thymus, prostate, carcinoid, urethra, non-small cell lung	1 each

Abbreviation: ECOG; Eastern Cooperative Oncology Group.

Table 2. BAY 12-9566 Dose Escalation Scheme

BAY 12-9566 Dose Schedule	No. of Patients			No. of Courses
	New	De-Escalated to Level	Total	
100 mg once daily	3	–	3	4
400 mg once daily	3	–	3	8
400 mg twice daily	3	1	4	12
400 mg thrice daily	6	1	7	13
400 mg four times daily	3	–	3	5
800 mg twice daily	3	–	3	5
TOTAL	21	–	–	47

biologically relevant concentrations, increased proportionately less than increasing BAY 12-9566 doses, and seemed to plateau as total daily BAY 12-9566 doses were increased.

Toxicity

BAY 12-9566 was well tolerated at all dose schedule levels evaluated. The most common adverse events were nausea and/or vomiting (13 patients), headache (seven patients), and fatigue (seven patients), although these complaints were attributed, in part, to the underlying malignant disease and/or concomitant medications in most cases. These effects were mild to moderate (grades 1 and 2) and managed successfully with nonopioid analgesics, typically acetaminophen or nonsteroidal anti-inflammatory medications and phenothiazine antiemetic medications. These adverse effects were not related to the BAY 12-9566 dose schedule or treatment duration. Other less common ($\leq$ two patients each), mild to moderate complaints possibly related to BAY 12-9566 included abdominal cramping, diarrhea, peripheral edema, mouth soreness, pruritus, myalgia, arthralgia, and hot flashes. These effects were not related to any particular BAY 12-9566 dose schedule and resolved after either no or minimal symptomatic treatment.

The most common biochemical abnormalities noted during the study were elevations in serum concentrations of hepatic transaminases and/or bilirubin and hypophosphatemia. A heavily pretreated 33-year-old man with an osteosarcoma and liver metastases experienced an isolated elevation in total serum bilirubin. The patient's total serum bilirubin increased from a pretreatment value of 0.8 mg/dL to a peak value of 1.5 mg/dL on day 18 of his first course of treatment with BAY 12-9566 at the 400-mg three times daily dose level. Concomitant elevations in hepatic transaminases and/or alkaline phosphatase were not noted. This modest, albeit grade 3, toxicity resolved completely within 5 days after treatment was discontinued and did not recur after resumption of BAY 12-9566 at a reduced dose of 400 mg twice daily. Five other subjects experienced isolated, grade 1 and 2 elevations in AST and ALT at most dose schedule levels, which may have been related to the study drug. Hypophosphatemia, which was noted in 12 patients at some time during treatment, was not associated with any other specific symptom, physical manifestation, or biochemical abnormality and was readily corrected after oral treatment with inorganic phosphorous.

Hematologic effects of potential clinical significance were uncommon. Albeit uncommon, thrombocytopenia was the principal hematologic effect of BAY 12-9566. Although absolute decrements in platelet counts were common, clinically significant reductions in platelets to less than 100,000/μL were noted in only four subjects. The most profound event was experienced by a 73-year-old heavily pretreated woman with metastatic breast cancer whose platelet count decreased from a pretreatment level of 242,000/μL to 39,000/μL on day 15 of her second course of BAY 12-9566 at the 400-mg three times daily dose level. Moderate anemia was also evident. The study drug was discontinued, and treatment was never restarted because of tumor progression. Additionally, a 54-year-old heavily pretreated woman with a widely metastatic malignant carcinoid neoplasm developed grade 2 thrombocytopenia (nadir platelet count, 69,000/μL) during her first course of BAY 12-9566 at the 400-mg four times daily dose level. She also experienced intermittent, albeit persistent, nausea and vomiting, which led to a BAY 12-9566 dose level reduction to 400 mg three times daily. During three subsequent courses of BAY 12-9566 at this dose level, her platelet counts ranged from 61,000/μL to 100,000/μL, and nausea and vomiting were seldom experienced. Two other heavily pretreated patients developed grade 1 thrombocytopenia (nadir platelet counts, 83,000/μL and 88,000/μL) at some time during their treatment with BAY 12-9566 at the 400-mg three times daily and 400-mg four times daily dose levels. Anemia, mild to moderate in severity, occurred occasionally and required an RBC transfusion in only one individual. WBC depressions were not noted.

Studies of MMPs in Plasma

Plasma concentrations of total MMP-2 were relatively stable from one sampling time to the next, whereas variability of total MMP-9 was somewhat greater. However, no consistent fluctuations in levels of MMP-2 and MMP-9 were noted at any dose level. The percent changes in plasma concentrations of total MMP-2 and MMP-9 on day 29 compared with pretreatment levels as a function of total daily BAY 12-9566 dose are displayed in Fig 2A and 2B. No relationships were apparent between the percent changes in MMP-2 and MMP-9 and total daily dose (r^2 = .014, P = .66 and r^2 = .019, P = .59, respectively). Substantial variability from one sampling time to the next, with no discernible pattern of fluctuation over the treatment course, was also noted for TIMP-2; however, the percent change in TIMP-2 on day 29 compared with pretreatment was moderately related to total daily BAY 12-9566 dose (r^2 = .50, P = .046) (Fig 2C). Active MMP-9 was not detected (ie, below the lower limits of assay detection) in any plasma sample.

Pharmacologic Studies

BAY 12-9566 plasma concentrations peaked 4 to 8 hours after treatment. C_{ss} values from plasma sampled before treatment on days 15, 29, and 57 were similar. A scatterplot

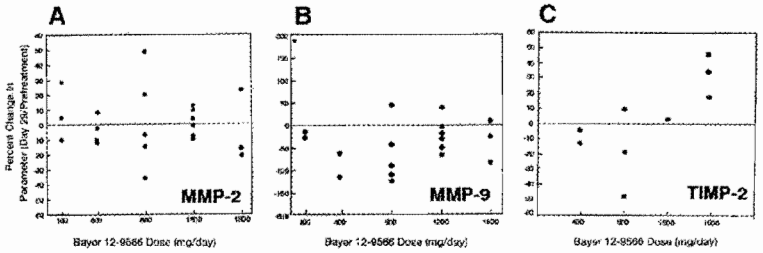


Fig 2. Scatterplots depicting percent change in MMP or TIMP on day 29 compared with pretreatment as a function of the total daily dose of BAY 12-9566: (A) MMP-2 (r^2 = .014, P = .66); (B) MMP-9 (r^2 = .019, P = .59); and (C) TIMP-2 (r^2 = .50, P = .046). (● = individual patient values).

of individual, as well as mean (± SD), plasma C_{ss} values from samples obtained before dosing on day 15 of the first course as a function of the dose schedule of BAY 12-9566 is shown in Fig 3. C_{ss} values increased less than proportionately as the daily BAY 12-9566 dose was increased within the dose range of 100 to 1,600 mg/d. Plasma C_{ss} averaged 35.77 ± 4.16 μg/mL (100 mg once daily), 86.80 ± 8.99 μg/mL (400 mg once daily), 113.25 ± 21.55 μg/mL (400 mg twice daily), 115.39 ± 39.19 μg/mL (400 mg three times daily), 105 ± 43.08 μg/mL (400 mg four times daily), and 141.86 ± 53.57 μg/mL (800 mg twice daily). BAY 12-9566 plasma C_{ss} values averaged 121.99 ± 48.23 μg/mL in patients treated with a total daily BAY 12-9566 dose of 1,600 mg/d. Except for C_{ss} values at the 100 mg/d dose level that were significantly less than those at all other dose levels (Student's t test; $P < .05$), there were no significant differences in plasma C_{ss} values resulting from treatment with BAY 12-9655 at daily doses of 400, 800, 1,200, and 1,600 mg. However, the numbers of patients at each dose schedule level were small. No relationships between BAY 12-9566 plasma C_{ss} and changes in plasma concentrations of total MMP-2 and MMP-9 levels were evident.

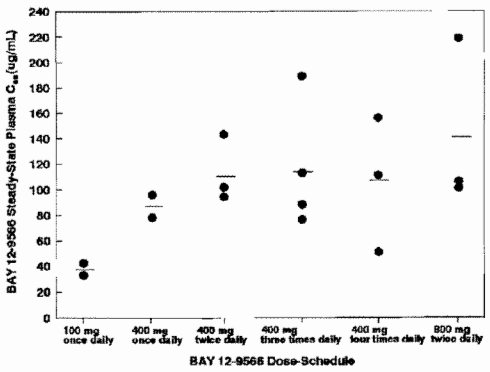


Fig 3. Scatterplot depicting BAY 12-9566 C_{ss} as a function of BAY 12-9566 dose schedule; (●) individual patient values, (—) mean values. Plasma C_{ss} values averaged 35.77 ± 4.16 μg/mL (100 mg once daily), 86.80 ± 8.99 μg/mL (400 mg once daily), 113.25 ± 21.55 μg/mL (400 mg twice daily), 115.39 ± 39.19 μg/mL (400 mg three times daily), 105 ± 43.08 μg/mL (400 mg four times daily), and 141.86 ± 53.57 μg/mL (800 mg twice daily). Plasma C_{ss} values averaged 121.99 ± 48.23 μg/mL in patients treated with a total daily BAY 12-9566 dose of 1,600 mg/d.

DISCUSSION

MMPs play important roles in tumor growth, invasion, and metastasis, and represent logical strategic targets for anticancer therapeutic development.[1-4,23] BAY 12-9566 is a potent and selective inhibitor of MMP-2, MMP-3, and MMP-9, which are associated with tumor invasiveness and metastasis.[1-11] The rationale for developing selective inhibitors of MMPs is that these agents may exhibit higher therapeutic indices than the first generation of nonspecific MMP inhibitors. This reasoning is based on the hypothesis that musculoskeletal toxicity is related to the nonspecific inhibition of the collagenases MMP-1 and MMP-13, which may not be as important targets as MMP-2, MMP-3, and MMP-9 in treating malignant diseases.[1-11,20,23,33,34]

Inflammation of the tendons and ligaments, which is the principal toxicity of the first generation nonspecific oral MMP inhibitor marimastat (British Biotech, Inc, Oxford, United Kingdom), was not observed in animal toxicology studies of BAY 12-9566.[4,20,23,34,35] Similarly, musculoskeletal effects were not noted in patients with advanced solid malignancies who were treated with BAY 12-9566 in the present study or in other early clinical investigations with BAY 12-9566.[36-38] In fact, there were no consistent adverse effects or an unacceptably high incidence of DLTs to preclude dose escalation of BAY 12-9566 on the daily treatment schedules evaluated in the present study. The most common adverse effects were headache, nausea, vomiting, thrombocytopenia, elevations of hepatic transaminases, and hypophosphatemia. These toxicities were neither dose- nor concentration-dependent, were generally mild to

moderate in severity, were and self-limiting and/or responsive to conservative measures of treatment. Unfortunately, the refractory nature of the malignant neoplasms of the patients in phase I studies, in general, preclude ascertaining much relevant information about the long-term effects of BAY 12-9566 and other cytostatic agents that have required protracted administration for optimal efficacy in preclinical studies. However, the limited experience with the four individuals who were treated for at least 4 months in the present study suggests good long-term tolerance of BAY 12-9566 on a protracted divided daily dosing schedule.

Because BAY 12-9566 and other MMP inhibitors may be combined with cytotoxic agents in many disease settings,[4,30] both acute and chronic overlapping toxicities may occur, particularly if BAY 12-9566 is administered in combination with agents that commonly induce thrombocytopenia and/or hepatotoxicity. This concern is only speculative at this juncture, and, in fact, the preliminary clinical results with BAY 12-9566 suggest a low likelihood for clinically relevant toxicologic interactions with several commonly used cytotoxic anticancer agents.[4,30] It may be prudent, however, to evaluate the potential for both toxicologic and pharmacokinetic drug interactions in the phase I setting before disease-directed evaluations of BAY 12-9566–based combination chemotherapy are performed.

The decision to terminate further dose escalation in the present study was based on progressively smaller increases in plasma C_{ss} values as the daily dose of BAY 12-9566 was increased from 100 to 1,600 mg and the plateau effect of plasma C_{ss} values at the higher doses. Mean plasma C_{ss} values increased from 35.77 ± 4.16 μg/mL to 86.80 ± 8.99 μg/mL as the total daily dose of BAY 12-9566 was increased from 100 to 400 mg. This situation of diminishing pharmacologic returns was much more pronounced at the higher dose levels; plasma BAY 12-9566 C_{ss} values averaged 113.25 ± 21.55 μg/mL, 115.39 ± 39.19 μg/mL, and 121.99 ± 48.23 μg/mL after treatment with total daily doses of 800, 1,200, and 1,600 mg, respectively. Although the lack of individual plasma concentration time plots at all dose levels precludes drawing definite conclusions regarding the etiology of this behavior, these results suggest that the absorption of BAY 12-9566 is a saturable process. Based on the pharmacologic profile, the acceptable toxicologic profile associated with protracted dosing at the 1,600-mg/d dose level and the nearly identical toxicologic and pharmacologic profiles associated with four times daily and twice daily dosing schedules, an 800-mg twice daily dosing schedule is recommended for further disease-directed evaluations. However, it must be acknowledged that all BAY 12-9566 dose schedules with total daily doses of 800, 1,200, and 1,600 mg/d in this study resulted in similar C_{ss} values, although the statistical power of such comparisons is low because of the small numbers of patients treated at each dose level. Therefore, it is possible that all of these dose schedules may result in similar functional and therapeutic effects.

It is encouraging that plasma C_{ss} values were at least two to three orders of magnitude higher than the K_i values reported for MMP-2, MMP-3, and MMP-9.[24] Although projections about the use of deriving optimal therapeutic doses of compounds by comparing pharmacologic parameters achieved in plasma with in vitro data may be misleading, the sheer magnitude of the plasma C_{ss} values achieved in the present study negates, at least in part, concerns that the pharmacologic behavior of BAY 12-9566 in the plasma compartment may not accurately reflect drug behavior in peripheral tissues and tumors. For example, differences between protein concentrations in preclinical studies and human plasma and interspecies differences in the magnitude and avidity of protein binding may result in difficulties gauging the relevance of any particular plasma C_{ss} value, as well as using plasma C_{ss} to guide dosing recommendations. For BAY 12-9566, the absolute magnitude of plasma C_{ss} values achieved in patients relative to MMP K_i values determined in vitro is much less impressive, even after the potential influence of high protein binding (99.9%) is taken into account.[31] However, the relationships between free drug concentrations and drug effect is not known.

The exploratory studies of MMP-2, MMP-9, and TIMP-2 in the present trial were undertaken to identify readily assessable and quantifiable parameters that reflect MMP inhibition. Hypothetically, such markers may be used to monitor MMP activity, determine the optimal dose schedule of MMP inhibitors, detect disease progression, and quantify responsiveness to therapy.[2,4,13,16,20,23,39,48] However, despite achieving biologically relevant plasma concentrations of BAY 12-9566, there were no consistent effects of BAY 12-9566 on plasma concentrations of MMP-2, and MMP-9. On the other hand, the percent decrement in TIMP-2 was moderately related to the total daily dose of BAY 12-9566. The mechanism accounting for this possible relationship is not known, but the overall direction of the relationship is somewhat paradoxical because a compensatory decrease in TIMP-2 might have been expected after inhibition of MMP-2.

Nearly identical results have been reported using enzyme zymography to measure plasma concentrations of MMP-2 and MMP-9 in phase I trials of the MMP inhibitors marimastat and batimastat (BB-94; British Biotech, Inc, Oxford, United Kingdom).[4,20,23,46] The preliminary results of exploratory studies measuring plasma concentrations of vascular endothelial growth factor and basic fibroblast

growth factor and urinary levels of pyridinoline and deoxypyridinoline in other phase I studies of BAY 12-9566 also revealed no consistent patterns.[36] However, these negative results do not absolutely indicate that MMP inhibitors are incapable of affecting relevant subcellular targets in humans. There may be substantial differences in the activities of MMP-2, MMP-9, and TIMP-2 between peripheral tissues and plasma, and, therefore, inferences regarding these proteins in tumors based on measurements in plasma may be inaccurate. In addition, little is known about the relationships between the total and activated forms of MMPs and TIMPs in both the plasma and tissue compartments.[1-4,40,47] Still, it is conceivable that some tumor types are more likely to produce readily quantifiable levels of activated MMPs than others, and attempts to identify reliable surrogates of effect should continue in disease-directed studies and more homogenous patient populations.

Although the recommended dose of BAY 12-9566 for subsequent disease-directed studies, 800 mg twice daily, is based, in part, on the achievement of biologically relevant pharmacologic end points and the leveling off of C_{ss} values in the higher range of BAY 12-9566 doses evaluated in this study, optimal dose selection in phase I studies may not be as clear for other MMP inhibitors and other classes of cytostatic agents. In addition to guiding optimal dose schedule selection, the identification of readily assessable surrogate markers of biologic activity will likely facilitate the screening of novel MMP inhibitors and cytostatic agents for clinically relevant activity in the phase II setting before initiating resource-intensive phase III evaluations. Although a somewhat loose notion regarding the potential usefulness of such agents may be obtained by comparing end points such as progression-free and overall survival in phase II studies with historical data, there are many potential flaws inherent in such nonrandomized approaches. The availability of validated and readily quantifiable surrogates of target effect is likely to facilitate clinical screening and enhance decision making about the potential use of novel therapeutics in early developmental phases. However, because reliable surrogates of MMP inhibition are not yet available, and, given the many potential flaws of nonrandomized phase II screening studies to assess the antiproliferative effects of such cytostatic agents, randomized phase III evaluations in relevant clinical settings may be the next most logical developmental step for BAY 12-9566, and such studies are currently in progress.

REFERENCES

1. Matrisian LM: The matrix-degrading metalloproteinases. Bioessays 14:455-463, 1992
2. Chambers AF, Matrisian LM: Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 89:1260-1270, 1997
3. Birkedal-Hansen H, Moore WG, Booden MK, et al: Matrix metalloproteinases: A review. Crit Rev Oral Biol Med 4:197-250, 1993
4. Denis LJ, Verweij J: Matrix metalloproteinase inhibitors: Present achievements and future prospects. Invest New Drugs 15:175-185, 1997
5. Stetler-Stevenson WG, Liotta LA, Kleiner DE Jr: Extracellular matrix 6: Role of matrix metalloproteinases in tumor invasion and metastases. FASEB J 7:1434-1441, 1993
6. Stetler-Stevenson WG, Krutzsch HC, Liotta L: Tissue inhibitor of metalloproteinase (TIMP-2): A new member of the metalloproteinase inhibitor family. J Biol Chem 264:17374-17378, 1989
7. Leco KJ, Khokha R, Pavloff N, et al: Tissue inhibitor of metalloproteinases-3 (TIMP-3) as an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues. J Biol Chem 269:9352-9360, 1994
8. Liotta LA, Stetler-Stevenson WG: Tumor invasion and metastasis: An imbalance of positive and negative regulation. Cancer Res 51:5054s–5059s, 1991 (suppl 18)
9. Tryggvason K, Hoyhtya M, Pyke C: Type IV collagenase in invasive tumors. Breast Cancer Res Treat 24:209-218, 1993
10. Liotta LA, Thorgeirsson UP, Garbisa S: Role of collagenases in tumor cell invasion. Cancer Metastasis Rev 3:361-372, 1984
11. Ray JM, Stetler-Stevenson WG: The role of metalloproteinases and their inhibitors in tumour invasion, metastasis, and angiogenesis. Eur Respir J 7:2062-2072, 1994
12. Khokha R, Martin DC, Fata JE: Utilization of transgenic mice in the study of matrix degrading proteinases and their inhibitors. Cancer Metastasis Rev 14:97-111, 1995
13. Watson SA, Morris TM, Robinson G, et al: Inhibition of organ invasion by the matrix metalloproteinase inhibitor batimastat (BB-94). Cancer Res 56:2815-2822, 1996
14. Bernhard EJ, Gruber SB, Muschel RJ: Direct evidence linking expression of matrix metalloproteinase 9 (92 kDa gelatinase/collagenase) to the metastatic phenotype in transformed rat embryo cells. Proc Natl Acad Sci USA 91:4293-4297, 1994
15. Zeng ZS, Guillem JG: Distinct pattern of matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase I mRNA expression in human colorectal cancer and liver metastases. Br J Cancer 67:1126-1131, 1993
16. Zeng ZS, Huang Y, Cohen AM, et al: Prediction of colorectal cancer relapse and survival via tissue RNA levels of matrix metalloproteinase-9. J Clin Oncol 14:3133-3140, 1996
17. Friedman R, Toth M, Pena D, et al: Activation of progelatinase B (MMP-9) by gelatinase A (MMP-2). Cancer Res 55:2528-2555, 1995
18. Liotta LA, Steeg PS, Stetler-Stevenson WG: Cancer metastasis and angiogenesis: An imbalance of positive and negative regulation. Cell 64:327-336, 1991
19. Ray JM, Stetler-Stevenson WG: Gelatinase A activity directly modulates melanoma cell adhesion and spreading. EMBO J 14:908-917, 1995
20. Wojtowicz-Praga S, Torri J, Johnson M, et al: Phase I trial of marimastat, a novel matrix metalloproteinase inhibitor, administered orally to patients with advanced lung cancer. J Clin Oncol 16:2150-2156, 1998

21. Moutsiakis D, Mancuso P, Krutzsch H, et al: Characterization of metalloproteinases and tissue inhibitors of metalloproteinases in human plasma. Connective Tissue Res 28:213-230, 1992

22. Wojtowicz-Praga SM, Dickson RB, Hawkins MJ: Matrix metalloproteinase inhibitors. Invest New Drugs 15:61-75, 1997

23. Rasmussen HS, McCann PP: Matrix metalloproteinase inhibition as a novel anticancer strategy: A review with special focus on batimastat and marimastat. Pharm Ther 75:69-75, 1997

24. Hibner B, Card A, Flynn C, et al: BAY 12-9566, a novel, biphenyl matrix metalloproteinase inhibitor, demonstrates anti-invasive and anti-angiogenic properties. Proc Am Assoc Cancer Res 39:302, 1998 (abstr 2063)

25. Hibner B, Bull C, Flynn C, et al: Activity of the matrix metalloproteinase inhibitor BAY 12-9566 against murine subcutaneous and metastatic in vivo models. Ann Oncol 9:75, 1998 (suppl 2)

26. Bull C, Flynn C, Eberwein D, et al: Activity of the biphenyl matrix metalloproteinase inhibitor BAY 12-9566 in murine *in vivo* models. Proc Am Assoc Cancer Res 39:302, 1998 (abstr 2062)

27. Gatto C, Rieppi M, Borsotti P, et al: Anti-angiogenic activity of BAY 12-9566, an inhibitor of matrix metalloproteinase. Ann Oncol 9:74, 1998 (suppl 2)

28. Flynn C, Bull C, Eberwein D, et al: Anti-metastatic activity of BAY 12-9566 in a human colon carcinoma HCT116 orthotopic model. Proc Am Assoc Cancer Res 39:301, 1998 (abstr 2057)

29. Flynn C, Bull C, Matherne C, et al: Anti-invasive and anti-metastatic activity of the novel MMP inhibitor BAY 12-9566 in subcutaneous and orthotopic models using the human colon carcinoma, HCT 116. Ann Oncol 9:75, 1998 (suppl 2)

30. Clemens GR, Detzer K, Bomhard E, et al: Preclinical drug safety profile for the anti-metastatic matrix metalloproteinase inhibitory agent BAY 12-9566. Ann Oncol 9:74, 1998 (suppl 2)

31. Shah A, Sundaresan P, Humphrey R, et al: Comparative pharmacokinetics of BAY 12-9566, a metalloproteinase inhibitor, in healthy volunteers and cancer patients. Proc Am Assoc Cancer Res 39:521, 1998 (abstr 3547)

32. Brubaker WF, Perrino P, Wu W, et al: Pharmacokinetics of BAY 12-9566 in animals: Species comparison. Proc Am Assoc Can Res 40:391, 1999 (abstr 2589)

33. Agarwal VK, Rose DL, Krol GJ: Quantitative HPLC analysis of 4-[4-4-(chlorophenyl)phenyl]-4-oxo-2S-(phenylthiomethyl) butanoic acid (BAY 12-9566), a metalloproteinase inhibitor and its metabolites in human plasma. J Liq Chromo Rel Technol 22:1893-1906, 1999

34. Neumanaitis J, Poole C, Primrose J, et al: Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: Selection of a biologically active and tolerable dose for longer-term studies. Clin Can Res 45:1101-1119, 1998

35. Millar AW, Brown PD, Moore J, et al: Results of single and repeat dose studies of the oral matrix metalloproteinase inhibitor marimastat in healthy male volunteers. Br J Clin Pharmacol 45:21-26, 1998

36. Erlichman C, Adjei A, Alberts, S, et al: Phase I study of BAY 12-9566: A matrix metalloproteinase inhibitor. Proc Am Soc Clin Oncol 17:217a, 1998 (abstr 837)

37. Grochow LB, O'Reilly S, Humphrey R, et al: Phase I and pharmacokinetic study of the matrix metalloproteinase inhibitor BAY 12-9566. Proc Am Soc Clin Oncol 17:213a, 1998 (abstr 822)

38. Goel R, Hirte H, Goel R, et al: Phase I study of the matrix metalloproteinase inhibitor (MMPI) BAY 12-9566 in patients with advanced cancer. Proc Am Soc Clin Oncol 17:217a, 1998 (abstr 840)

39. Azzam HS, Arand G, Lippman ME, et al: Association of MMP-2 activation potential with metastatic progression in human breast cancer cell lines independent of MMP-2 production. J Natl Cancer Inst 85:1758-1764, 1993

40. Zucker S, Lysik RM, Zarrabi HM, et al: Plasma assay of matrix metalloproteinases (MMPs) and MMP-inhibitor complexes in cancer: Potential use in predicting metastasis and monitoring treatment. Ann NY Acad Sci 732:248-262, 1994

41. Levy TD, Cioce V, Sobel ME, et al: Increased expression of the Mr 72,000 type IV collagenase in human colonic adenocarcinoma. Cancer Res 51:439-444, 1994

42. Zucker S, Lysik RM, Zarrabi MH, et al: M_r 92,000 type IV collagenase is increased in plasma of patients with colon and breast cancer. Cancer Res 53:140-146, 1993

43. Brown PD, Bloxidge RE, Stuart NSA, et al: Association between expression of activated 72-kilodalton gelatinase and tumor spread in non-small cell lung carcinoma. J Natl Cancer Inst 85:574-578, 1993

44. Iizasa T, Fujisawa T, Suzuki M, et al: Elevated levels of circulating plasma matrix metalloproteinase 9 in non-small cell lung cancer patients. Clin Cancer Res 5:149-153, 1999

45. Moses M, Wiederschain D, Loughlin KR, et al: Increased incidence of matrix metalloproteinases in urine and cancer patients. Cancer Res 58:1395-1399, 1998

46. Wojtowicz-Praga S, Low J, Marshall J, et al: Phase I trial of a novel matrix metalloproteinase inhibitor batimastat (BB-94) in patients with advanced cancer. Invest New Drug 14:193-202, 1996

47. Moutsiakis D, Mancuso P, Krutzsch H, et al: Characterization of metalloproteinases and tissue inhibitors of metalloproteinases in human plasma. Connective Tissue Res 28:213-230, 1992

48. Zucker S, Lysik RM, Zarrabi MH, et al: Type IV collagenase/gelatinase (MMP-2) is not increased in plasma of patients with cancer. Cancer Epidemiol Biomarkers Prev 1:475-479, 1992

A-291518:
Matrix Metalloproteinase Inhibitor for Treatment of Cancer

I. Executive Summary

Abbott's matrix metalloproteinase (MMP) inhibitor program represents a novel therapeutic class with the potential to alter the way that cancer is treated by preventing or modifying disease progression and/ or metastases. This more "chronic" approach to therapy has the potential to transform cancer into a disease that patients live with, much like the effect of HIV protease inhibitors on patients with AIDS. It also has the potential to expand the cancer market significantly by increasing the average length of treatment and expanding the pool of patients eligible to receive therapy.

The MMPs comprise a family of enzymes that degrade a wide range of matrix protein substrates. High expression of these enzymes occurs in cancer and is associated with the ability of tumors to grow, invade, develop new blood vessels and metastasize. Experimental evidence suggests that gelatinase A and gelatinase B are particularly important in tumor progression. The Project Team has therefore targeted gelatinase selective inhibitors for the treatment of cancer.

Another reason for targeting highly gelatinase selective MMP inhibitors relates to the side effect profile exhibited by broad spectrum agents like marimastat. Chronic administration of marimastat causes severe joint pain and stiffness which precludes the examination of higher doses in clinical trials. Results from Phase III studies with marimastat have been both positive (gastric cancer) and negative (pancreatic cancer) and suggest that MMP inhibitors are more likely to benefit patients in the earlier stages of disease progression.

Toxicity studies with ABT-770, Abbott's first MMP inhibitor development candidate, revealed a number of adverse effects which occurred at drug exposures only several fold higher than that necessary for efficacy in animal models. Consequently, a decision was made to abandon further development of ABT-770 in lieu of A-291518, a member of Abbott's biaryl ether retrohydroxamate series of inhibitors. The MMP selectivity profile exhibited by A-291518 distinguishes it from ABT-770 and competitor's compounds. A-291518 possesses sub-nanomolar potencies versus gelatinase B, an improvement of 200-fold over ABT-770. A-291518 is also substantially more selective for the inhibition of the gelatinases over fibroblast collagenase than prinomastat, suggesting that it may avoid mechanism-based joint effects. A-291518 is a stable crystalline solid which can be synthesized in six steps (25% overall yield) from commercial starting materials.

A-291518 demonstrates antitumor activity equal or superior to ABT-770 and prinomastat. Inhibition of tumor growth is dose dependent in both syngeneic and xenograft models. Blood concentrations of A-291518 necessary to achieve minimal efficacy in the B16 melanoma tumor growth model are one-tenth that of ABT-770. A-291518 is also effective in blocking vessel formation in a mouse model of angiogenesis.

A-291518 gives rise to sustained plasma concentrations following single oral dosing in monkeys, dogs and rats. Bioavailabilities range between 68 and 93% depending on formulation and species. Multiple metabolites are produced after repeated oral dosing, some reaching plasma concentrations in excess of parent drug. Most of the metabolites result from modification of the retrohydroxamate moiety, although their relative amounts varies with gender and species.

The safety profile exhibited by A-291518 is an improvement over ABT-770. It displays no meaningful effects in genotoxicity, cytotoxicity and ligand binding assays and its cardiovascular effects in dogs are unremarkable. Toxicity studies in rats and monkeys revealed none of the debilitation and lethality seen with ABT-770. Tissues from these animals revealed no evidence of phospholidiposis which likely reflects the reduced tissue burden of metabolites produced by A-291518 relative to ABT-770. The plasma concentrations generated by A-291518 in rat toxicity studies are at least 20-fold higher than those necessary to produce efficacy in cancer animal models. A-291518 is therefore a compelling successor to ABT-770 with the potential to demonstrate antitumor effects superior to the MMP inhibitors currently undergoing clinical trials.

II. Medical Need

Cancer is projected to be the number one cause of death in industrialized nations by the year 2003. Forty percent of all Americans will develop cancer in their lifetime. As the population in the U.S. grows older, the incidence rates for various cancers will continue to rise. Cancer is not a single disease, but includes more than 100 different histologic subtypes, which have at their core, an imbalance between cell growth and apoptosis. Treatments of breast, lung, and prostate cancers account for more than 50 percent of the direct medical costs of cancer therapies. For most cancers, physician satisfaction with current therapies is low. In many cases, current treatment options are worse than the underlying disease. Unmet needs in the cancer market center around inadequate efficacy and excessive toxicity of current treatments. While unmet need varies by tumor type and stage, cancer is still usually a terminal disease.

III. Market Overview

A. Oncology Market

Cancer remains the second leading cause of death in the United States, Europe and Japan. This year about 552,200 Americans are expected to die of cancer, more than 1,500 people a day. In 2000, about 1.2 million new cancer cases are expected to be diagnosed in the U.S. Trends are not radically dissimilar outside the U.S. With the aging population as a key driving force, the incidence of cancer will continue to escalate from a figure of 10MM in 1996 to approximately 15MM by 2015.

The worldwide cytotoxic and hormonal cancer therapies market is a fragmented market, with only BMS and Zeneca holding a greater than 10% share of the market. Although the market is not concentrated, the field is highly competitive with more than 60 companies focused on the cancer research area. Considering market, clinical and patient dynamics factors, breast, colorectal, prostate and non-small-cell lung cancers are the most attractive targets for development. The growth of the oncology market is fueled by increasing disease incidence, new product entries, new therapeutic approaches, a growing adjunct therapy market (which increases the number of patients eligible for chemotherapy) and intensifying research competition. Table 1 summarizes the value of the current oncology market.

Table 1. Oncology Market

A. Sales by Market Segment ($ MM).

	1996 Sales	1997 Sales	1998 Sales	1999 Sales (est)	CAGR '96-'98
Hormone	4,414	4,784	4,884	5,000	5.2%
Cytotoxic	4,278	5,212	6,268	7,300	21.0%
Adjunctive	3,367	3,651	4,166	4,900	11.2%
Total	12,059	13,647	15,318	17,200	12.7%

Source: Data monitor/ IMS

B. Sales by Region ($ MM).

	1996 Sales	1997 Sales	1998 Sales	1999 Sales (est)	CAGR '96-'98
U.S.	5,564	6,276	7,422	8,500	15.5%
Ex-U.S.	6,495	7,370	7,896	8,700	10.3%

Source: Frost & Sullivan/ IMS

B. MMP Inhibitor Market Assessment

MMP inhibitors and other "cytostatic" therapies may alter the way cancer is treated by preventing or modifying the disease progression and/ or metastases. The goal of cytostatic therapy, to improve quality of life for the cancer patient, stabilize the disease and transform cancer from a disease to a chronic condition, has been compared to HIV protease inhibitors for treatment of AIDS. Consequently, these agents have the potential to expand the cancer market significantly by increasing the average length of treatment and expanding the pool of patients eligible to receive therapy. An assessment of the MMP inhibitors market should consider the factors listed below.

1. Favorable Considerations

Product Usage: As "cytostatic" agents, MMP inhibitors will likely be used as a maintenance therapy in early disease or after primary therapy as a prophylactic process to prevent the spread of malignancy. While physicians have indicated that they would use MMP inhibitors initially in more refractory patients as follow-on or add-on to current best therapy (chemotherapy or surgery), with experience and clinical evidence, prophylactic use in early stage disease is envisioned.

Product Benefits/Efficacy: Physicians are anticipating improvements in time to tumor progression and suppression of metastases with cytostatic agents. Preclinical studies suggest that MMP inhibitors play a role in primary tumor growth and tumor angiogenesis. Positive results from competitive agents, such as marimastat in gastric cancer, support these mechanisms of action.

Side Effects: The proposed safety profile for MMP inhibitors (excluding joint toxicity) may enhance usage, since the dose limiting toxicity profiles of most cytotoxic agents has established a much lower hurdle for demonstrating a preferred profile. However, MMP inhibitors will be dosed chronically, therefore they may *have* to demonstrate a cleaner profile than cytotoxic agents to ensure compliance. If Abbott's compound is the 3rd or 4th MMP inhibitor to market, side-effect hurdles will be even higher for this compound. As a critical Go/ No Go decision point, the joint toxicity of this compound will be evaluated in an expanded Phase I multi-dose study.

Dosing: Oral therapies are acknowledged by physicians and patients as being more convenient to the patient. Chronic oral dosing may also reduce overall costs, as infusion support products and personnel would not be required.

COGS: Initial estimates on finished cost of drug suggest that drug costs will not be significant for this compound. With the pricing flexibility in the U.S. market, an 80-90% margin on this product is anticipated.

Off-label use: Off label use typically accounts for 30-60% of an oncology product's usage. Off-label use is driven by publication of clinical trial results in scientific journals, listing in key compendia and/ or a clinicians experience with the product. Therefore, development spending for off-label use is considerably less than spending required for regulatory approval for a given indication. However, promotion of these off-label uses can be limited.

2. Unfavorable Considerations

Competition: Abbott's MMP inhibitor could potentially be the 3rd or 4th to market, consequently it will need to demonstrate a meaningful clinical advantage over compounds that are in more advanced development. Strict Go/ No Go criteria will determine if this MMP inhibitor can meet these hurdles. If they cannot be met, the compound will not move forward.

Development/Regulatory: With a new class of compounds, there is not a clearly defined clinical development path or regulatory guidelines for reference. This hurdle is similar for all the competitive products, but increases the overall development risk profile for these agents. However, with several MMP inhibitors in late stage development, the development of Abbott's agent can be guided by competitors experiences.

Other Approaches: Other "cytostatic" approaches may present a competitive threat if they are used as substitutes. Due to the complexity of the pathogenesis of cancer, it is more likely that these agents will be used in combinations, but incremental benefits may become more difficult to demonstrate as the number of products and approaches increase. This will require additional studies, as these other classes become part of standard cancer treatment. However, this threat is not unique to this compound.

Pricing: The treatment of cancer is expensive, consequently the potential for pricing flexibility in this market is large. Yet as an oral therapy in the U.S. market, there may be additional downward price pressure for MMP inhibitors. There is also an increasing emphasis on cost-effectiveness studies that will need to be addressed in the development plan.

Dosing: In general, oral therapies are preferred by physicians and patients because of the convenience to the patient. However, this form may not be the best choice for patients who suffer from digestive system problems (vomiting, diarrhea, or severe nausea), cannot swallow, or have difficulty complying with dosing protocols due to dementia. Additionally, in the U.S. market there are several unique factors that currently do not favor oral therapies. Novel oral therapies are not currently reimbursed by Medicare, a significant payer for the oncology patient population. Also, 40-60% of a community oncologist's income is generated through the administration of intravenous drugs. An oral therapy would not be a source of revenue to the physician.

3. Sale Forecast

A sales forecast was developed for the U.S. and ex-U.S. markets for Abbott's MMP inhibitor. Table 2 provides the assumptions associated with the sales forecast given in Figure 1.

Table 2. MMP Inhibitor Sales Forecast Assumptions.

	U.S. Downside	U.S. Base	U.S. Upside	Ex-U.S. Downside	Ex-U.S. Base	Ex-U.S. Upside
Indications	2nd line breast 2nd line NSCL	2nd/ 1st line breast 2nd/ 1st line NSCL	2nd/ 1st line breast 2nd/ 1st line NSCL	2nd line breast 2nd line NSCL	2nd/ 1st line breast 2nd/ 1st line NSCL	2nd/ 1st line breast 2nd/ 1st line NSCL
Off-label	2nd line ovarian 2nd line pancreas	2nd/ 1st line ovarian 2nd/ 1st line pancreas	2nd/ 1st line ovarian 2nd/ 1st line pancreas	N/ A	N/ A	N/ A
Eligible patients (2009 in 000's)	92.2	174.2	174.2	51.6[a]	127[a]	127[a]
Cost/ Ptnt/ Yr	$7,500	$7,500	$10,000	$4,500	$4,500	$6000
All MMPI Patient Share	30%	40%	50%	45%	45%	45%
Abbott Patient Share	20%	25%	35%	22%	22%	29%
Peak Sales WAC	$96	$235	$430	-	-	-
Peak Sales AWP	$115	$282	$516	$98	$229	$382
After-Tax NPV	$36	$172	$356	$45	$119	$196

a. Ex-U.S. patient numbers are for 5 major European markets (Germany, France, U.K., Italy, Spain) only; sales for the rest of the world derived as a percent of European sales

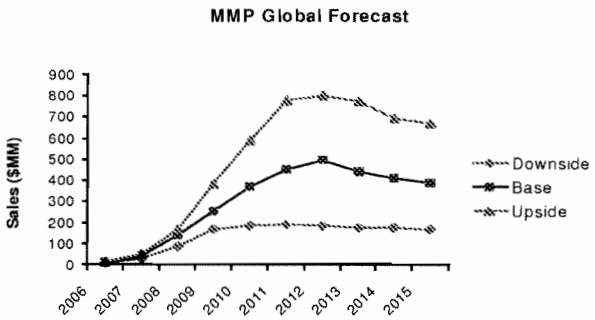


Figure 1. Global Sales Forecast for Abbott's MMP Inhibitor.

4. Commercial Recommendation

There continues to be a market opportunity for an improved MMP inhibitor. Current development compounds have less than ideal profiles. An MMP inhibitor which offers a

Exhibit A
Part 2

meaningful clinical benefit with a reduced side-effect profile or clinically significant improvements in efficacy with comparable side-effects represents a commercially attractive product. The clinical development plan will be designed to provide insight into these attributes. A key differentiation opportunity is lack of joint toxicity, which will be assessed in expanded multiple dose Phase I studies.

IV. Key Development Issues

A. Challenges in the Development of MMP Inhibitors

Examples of drugs designed for chronic treatment/ disease stabilization for cancer patients do not yet exist, consequently animal models have not been validated as predictive of responses in humans. There currently are a lack of accepted methods for determining the activity of cytostatic agents early in clinical trials. Traditional Phase II clinical endpoints such as tumor response rates are not appropriate for assessing the efficacy of MMP inhibitors since these compounds do not posses cytotoxic activity.[1] Indeed, few objective responses have been reported for the MMP inhibitors currently in clinical trials. Surrogate endpoints, such as the change in rate of rise of tumor markers, have been used to gauge the efficacy of MMP inhibitors, yet results from these studies have been difficult to interpret.[2] Changes in the degree of tumor encapsulation by fibrotic tissue may be one measure of drug activity in cancers where repeated biopsies are feasible.[3] In contrast to cytotoxic agents, dose escalation to the maximum tolerated dose may not be appropriate. In fact, avoiding inappropriately high doses will be particularly important for maintaining selective inhibition of the MMPs that mediate tumor progression over others whose inhibition may lead to joint toxicity (Section V.C). In the absence of a robust pharmacodynamic marker of MMP activity, the most appropriate criteria for defining doses for Phase II/ III trials will be targeting plasma concentrations associated with efficacy in preclinical models.

Since most patients have micrometastasis at diagnosis, it is naïve to think that MMPs inhibitors will be used solely as antimetastatic agents. Yet there is a reasonable possibility that these agents could slow or prevent the progression of micrometastases following treatment of the primary tumor. For that reason, MMP inhibitors will most likely be used in combination or immediately following conventional cytotoxic chemotherapy. Another key issue for the clinical assessment of MMP inhibitors relates to the selection of appropriate tumor types and patients. Based on the multiple mechanisms by which the MMPs facilitate tumor progression and their over-expression in wide range of tumor tissues (Section V.B.2), the development of inhibitors could potentially be geared toward many types of human cancers. Yet results from Phase III studies with marimastat (Section XV.A.1) suggest that MMP inhibitors may be most effective in patients with earlier stage and/ or less aggressive cancers. Consequently, establishing efficacy in terms of defined clinical endpoints (time to disease progression and/ or survival) is likely to require long-term or maintenance treatment.

B. Development Status of ABT-770

The first MMP inhibitor proposed by the Project Team for clinical development was ABT-770, a member of the retrohydroxamate class of inhibitors. Two-week toxicity studies with ABT-770 in rats and monkeys revealed a number of adverse effects including emaciation and lethality that tended to occur toward the end of the treatment period among high-dose animals. High concentrations of metabolites were detected, particularly in lung and liver tissue. Accordingly, the Transition Team delayed the initiation of a Phase I single-dose study in healthy volunteers (scheduled to begin October 1999) pending completion of one-month toxicity studies. Results from those studies reduced the therapeutic window for ABT-770 even further. In the monkey study (dosages of 20, 60 and 180 mg/ kg/ day), drug-related anorexia/ debilitation/ deaths were noted in the mid- and high-dose groups. Gastric lesions and extensive phospholipidosis was noted in all dosages. Rats treated with ABT-770 over a one month period gave rise to similar results; phospholipidosis was observed in numerous tissues and drug-related deaths occurred in high-dose animals. Based largely on the unknown cause of death and the resulting inadequate therapeutic ratio (Section XIII.G), a decision was made to discontinue further development of ABT-770 and to concentrate on the identification of a suitable backup compound.

V. Scientific Logic

The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases that degrade extracellular matrix components. Remodeling of the extracellular matrix is critical for tumor cell invasion, metastasis and angiogenesis. The expression and proteolytic activity of the gelatinases is elevated in tumor tissue and is associated with poor prognosis for a variety of malignancies. A reduction in tumor growth and metastasis is observed in gelatinase A and gelatinase B-deficient mice. Based on clinical results of competitor inhibitors, the Project Team has focused on compounds which inhibit the gelatinases in preference to fibroblast collagenase and related metalloproteinases such as TACE. This inhibition profile is intended to provide efficacy without causing mechanism-based joint effects.

A. Overview of the Matrix Metalloproteinases

The MMPs are a family of tightly regulated enzymes involved in the catabolic aspect of remodeling and maintenance of normal tissue.[4-9] Most are excreted as inactive proenzymes that are activated extracellularly by serine proteases or other MMPs. These activated enzymes are inhibited by secreted proteins known as tissue inhibitors of metalloproteinases (TIMPs).[10] The balance between activated MMPs and TIMPs determines the overall MMP proteolytic activity and, consequently, the extent of extracellular matrix turnover. Alteration of this balance in favor of excess enzyme activity contributes directly to cancer progression as well as other diseases involving matrix degradation including arthritis[11] and macular degeneration[12] (Section XIV).

The MMPs can be divided into four families based on similarities of their domain structure (Table 3).[6] The smallest MMP, matrilysin (PUMP-1, MMP-7), is the only member of the Minimal Domain family and consists of a signal sequence, a prodomain with a "cysteine switch" that is removed during activation, and a zinc containing catalytic domain.[13] This enzyme cleaves several substrates including proteoglycan, laminin, and fibronectin. The addition of a hemopexin binding domain connected by a hinge region to the *C*-terminus of the catalytic domain characterizes the Hemopexin Domain family. The collagenases, named because of their unique ability to cleave fibrillar collagen, are members of this family and include fibroblast collagenase (interstitial collagenase, MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13). Metalloelastase (MMP-12) and stromelysin-1, -2 and -3 (MMP-3, -10, -11) also contain hemopexin domains. Stromelysin-3 contains a putative furin enzyme recognition sequence that may contribute to its secretion from cells in a catalytically active form. As the name implies, the Fibronectin Domain family of MMPs, including gelatinase A and B (72 kD gelatinase, MMP-2 and 92 kD gelatinase, MMP-9) contain fibronectin-like sequences within the catalytic domain and are capable of degrading a broad collection of matrix substrates including gelatin, type IV collagen and elastin. There are currently five family members of the Transmembrane Domain family of MMPs (MMP-14 through MMP-17) plus a recently discovered enzyme.[14] These enzymes contain a sequence that is capable of spanning the cellular membrane and are proposed to activate gelatinase A at the surface of cells.[15]

Table 3. Classification of MMPs and Substrates.

MMP Class	Synonym	Substrate
Minimal Domain		
MMP-7	matrilysin	gelatin, fibronectin, non-fibrillar collagen
Hemopexin Domain		
MMP-1	fibroblast collagenase	collagens I, II, III, VII, X
MMP-8	neutrophil collagenase	collagens I, II, III
MMP-13	collagenase-3	collagens I
MMP-12	metalloelastase	elastin
MMP-3	stromelysin-1	gelatins, proteoglycans, fibronectin
MMP-10	stromelysin-2	gelatins, fibronectin
MMP-11	stromelysin-3	laminin, serine protease inhibitors
MMP-19	no trivial name	not defined
MMP-20	enamelysin	amelogenin
Transmembrane Domain		
MMP-14	MT-MMP-1	pro-MMP-2
MMP-15	MT-MMP-2	not defined
MMP-16	MT-MMP-3	pro-MMP-2
MMP-17	MT-MMP-4	not defined
not yet assigned	MT-MMP-5	not defined
Fibronectin Domain		
MMP-2	gelatinase A	collagens IV, V, VII, X, fibronectin, gelatins
MMP-9	gelatinase B	collagens I, III, IV, V, gelatins

B. Role of MMPs in Cancer

1. Potential Mechanisms

The role of the MMPs in the progression of cancer involves several mechanisms. They mediate invasion and metastasis by providing a mechanism through which tumor cells can traverse basement membranes thereby gaining access to blood and lymphatic vessels. Evidence for this mechanism is based largely on the increased invasiveness of cell lines which overexpress the MMPs.[16, 17] Recent studies suggest that MMPs may mediate primary tumor growth.[18] This involves remodeling of the extracellular matrix in the vicinity of the primary tumor such that stroma-bound growth factors are liberated. Similarly, several MMPs have been shown to degrade insulin-like growth factor-binding protein-3 thereby enhancing growth factor bioavailability.[19]

The MMPs play an important role in tumor angiogenesis by facilitating the proliferation and migration of endothelial cells and the neovascularization of tumor tissues.[20-22] Gelatinase A expression and activation is induced in endothelial cells cultured in a three-dimensional type I collagen lattice. This is mediated by a complex formed between MT1-MMP and TIMP-2 at the cell surface and suggests that selected MMPs play a significant role in cell-extracellular matrix interactions.[23] Furthermore, inhibition of endothelial cell migration through various extracellular matrix components has been demonstrated with endogenous MMP inhibitors as well as anti-MMP antibodies.[24]

The mechanisms outlined above suggest several clear advantages of MMP inhibitors over traditional chemotherapeutic agents. Since MMP inhibitors are not necessarily cytotoxic, they are unlikely to be compromised by the intrinsic toxicities that limit both the effective dose and duration of treatment for current cytotoxic drugs. Furthermore, resistance to MMP inhibitor therapy should be minimal since most MMPs have been shown to be derived from genetically stable stromal cells.

2. Correlation between MMP Expression and Progression of Human Malignancy

Much of the experimental evidence that supports the association of MMPs with tumor progression and metastasis comes from studies demonstrating elevated concentrations of the MMPs in human tumor biopsies. Virtually all the MMPs have been detected in tumor tissue, and most have been detected in more than one tumor type. Although the pattern of enzyme expression varies from tumor to tumor, in general the number of different MMPs detected tends to increase with tumor progression and the concentration of individual MMPs tends to increase with increasing tumor stage. For example, increased gelatinase-A expression has been demonstrated in carcinomas of the pancreas[25], breast[26], ovary[27] and colon[28, 29] and fibroblast collagenase mRNA concentrations are higher in malignant head and neck tissue than in adjacent normal tissue.[30, 31] The detection of MMP mRNA directly in tumor specimens by *in situ* hybridization indicates that most MMPs are produced by stromal rather than neoplastic cells.[32] This implicates the release of cancer cell-derived factors as stimulators of stromal MMP activity and emphasizes the importance of tumor-stromal interactions in the progression of solid tumors.[33, 34]

Elevated MMP activity in human biopsies has been correlated with tumor behavior. Enhanced gelatinase activity is associated with a higher tumor grade in breast[26], bladder[35], pancreatic[36] and gastric cancers.[37] In colorectal cancer, tumor expression of fibroblast collagenase has been correlated with poor prognosis[38] and stromelysin-3 mRNA levels correlate with decreased patient survival in breast cancer.[39] Measurement of plasma and urine MMP concentrations, either alone or complexed with TIMP, has been examined as a noninvasive method for cancer diagnosis and monitoring.[40, 41]

While these studies provide useful correlative evidence in support of the role of MMPs in cancer, it is important to emphasize that only a subset of MMPs are likely to be involved in any particular disease state. This raises the question as to which MMPs are primarily involved in the progression of cancer and whether these MMPs differ from those responsible for normal physiological processes such as tissue remodeling. Despite these uncertainties, it is clear that some MMPs are associated with human cancers more often than others. A number of studies suggest that gelatinase A is particularly important in tumor progression. First, type IV collagen is a major component of basement membranes and is a particularly good substrate for the gelatinase A. Transfection of the C127 breast cancer cell line with gelatinase A, but not stromelysin, gives rise to an invasive and metastatic phenotype.[42] Gelatinase A appears to be an important contributor to angiogenesis since endothelial cell tube formation is increased in the presence of recombinant enzyme.[20] Activation of pro-gelatinase A is distinct from most pro-MMPs and involves the formation of a ternary complex between gelatinase A, TIMP-2 and MT-MMP-1 at the cell surface.[13, 43] Since pro-gelatinase A is mainly expressed by stromal cells, this suggests a mechanism by which tumors can use host derived MMPs to facilitate invasion. Consistent with this mechanism are recent results demonstrating the binding of gelatinase A to the surface of invasive cells via interaction with the adhesion molecule $\alpha v\beta 3$.[44]

Experiments using gelatinase A-deficient mice support the role of gelatinase A in tumor progression.[45] These mice display reduced tumor-induced angiogenesis and a suppression of intradermal tumor growth rates relative to normal mice. Lung colonization after intravenous injection of tumor cells was also suppressed emphasizing the importance of stromal-derived MMPs in tumor progression. Significantly, these mice showed no developmental abnormalities.

Numerous studies also support an important role for gelatinase B in tumor progression. Overexpression of pro-gelatinase B correlates with higher tumor grade and stage of disease in active bladder cancer.[46] Invasion of an ovarian cell line was substantially reduced in the presence of anti-catalytic gelatinase B antibody[47] and murine prostate carcinoma cells transfected with gelatinase B ribozyme were unable to form lung colonies in experimental metastasis models.[48] The importance of host-derived gelatinase B activity in tumor metastasis is highlighted by experiments using gelatinase B-deficient mice. The number of metastastic colonies in these mice fell by 59% relative to wild-type animals following intravenous injection

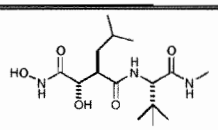
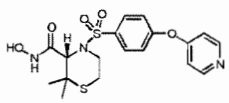
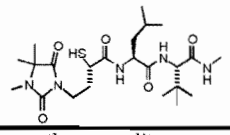
of Lewis lung carcinoma cells.[49] The putative role of gelatinase B in angiogenesis is bolstered by the observation that crossing Rip Tag mice (a strain of mice predisposed to developing pancreatic tumors) with gelatinase B-deficient animals resulted in reduced tumor burden in the off spring.[50] Analogous to gelatinase A, tumor cell surface localization of gelatinase B via binding to CD44 may regulate tumor invasiveness as well as angiogenesis.[51, 52]

While the gelatinases have historically been viewed as mediating only the progression of solid tumors, recent evidence implicates gelatinolytic activity in myeloproliferative malignancies. Both gelatinase A and gelatinase B have been shown to be up-regulated in bone marrow mononuclear cells from patients with acute and chronic myeloid leukemia.[53-55]

C. MMP Inhibitors

The MMP inhibitors field is competitive. Small molecule synthetic MMP inhibitors have been pursued by pharmaceutical companies since the late-1980's principally for the treatment of cancer and/ or arthritis.[56] A list of MMP inhibitors currently in advanced clinical development for the treatment of cancer is given in Table 4 and a comprehensive discussion of these compounds, as well as MMP inhibitors being developed for other indications is provided in Section XV. The most potent agents described to date are reversible, active site inhibitors that contain a functional group capable of binding the catalytic zinc atom. While the early inhibitors were designed based on the structure of MMP substrates and are therefore peptidic in nature (e.g. marimastat), screening hits have subsequently given rise to non-peptide inhibitors with superior pharmacokinetics.[57]

Table 4. MMP Inhibitors in Advanced Clinical Development

MMP Inhibitor Company	Structure	Development Status	Metalloproteinase Selectivity IC_{50} (nM)[a] MMP-1	MMP-2	TACE	Clinical Joint Toxicity
marimastat British Biotech/ Schering		Phase III	broad spectrum 0.78	0.41	1.8	yes
prinomastat Agouron/ Warner Lambert		Phase III	moderately gelatinase selective 5.7	0.048	7.9	yes
BMS-275291 Bristol-Myers Squibb/ Chirosciences		Phase II	broad MMP; no TACE (9)	(41)	(--)[b]	yes

a. IC_{50} values in parentheses are literature values.
b. Enzymatic assay data not available. Compound reported to lack inhibitory activity in a cellular assay of TNFα release.

One of the key issues in the MMP inhibitor field relates to whether broad spectrum inhibitors, active against a range of different enzymes, or selective inhibitors, targeted against a sub-set of MMPs, represent the optimal antitumor strategy. The compounds listed in Table 4 exhibit a range of MMP inhibition selectivities. Marimastat possesses broad spectrum metalloproteinase inhibition having essentially equivalent potencies against all the MMPs. It also inhibits related metalloproteinases such as TNFα-converting enzyme (TACE), a member of the adamalysin (ADAM) family.[58] Results from a number of clinical studies indicates that marimastat causes a dose-dependent joint toxicity typically described as arthralgia, myalgia and tendinitis occurring predominantly in the upper limbs.[59, 60] This usually begins in the small joints of the dominant hand and spreads to the arms and shoulders. In severe cases, upper body range

of motion is dramatically reduced (commonly referred to as "frozen shoulder") and nodules develop in the palms of the hands. While mild cases respond to analgesics, interruption of therapy for a period of approximately 2 weeks is necessary when the condition is less well tolerated.

Scientists at Agouron have expressed interest in developing highly gelatinase selective inhibitors as a means of avoiding joint toxicity.[61] However, prinomastat is only moderately selective (approximately 100-fold) for the inhibition of the gelatinases over fibroblast collagenase and TACE. While prinomastat has generally been well tolerated in clinical trials, doses of 10 mg twice daily and higher produce arthralgia after chronic dosing in cancer patients.[62, 63] As with marimastat, the incidence and rate of onset of joint effects seen with prinomastat are dose related; higher doses giving rise to greater incidence over a shorter time period.

The differences in chemical structures between marimastat and prinomastat suggests that the MMP inhibitor-induced joint toxicity is mechanism based, yet the exact cause has not been established and expert opinions on this issue vary. Tissue remodeling occurs regularly in the tendons and joints, so inhibition of enzymes involved in this process may be responsible. This implicates fibroblast collagenase (MMP-1) since it is produced by fibroblasts in connective tissue and is capable of degrading helical collagen, a major matrix component within joints and tendons.

Inhibition of metalloproteinases structurally related to the MMPs has also been hypothesized as the cause of joint toxicity. TACE has demonstrated "sheddase" activity through its ability to cleave a number of membrane-bound ligands including TNFα and TNFα receptors.[64] Alterations in the processing of these and other cell associated proteins may lead to dysregulated fibroblast function and ultimately to arthralgia and myalgia. The ADAM and MMP family of metalloproteinases have structurally similar active sites and marimastat has been shown to inhibit both TNFα and TNFα receptor release from THP-1 cells.[65] The clinical data presented thus far for BMS-275291 would seem to argue against TACE inhibition as mediating joint toxicity. This compound possesses broad spectrum MMP inhibition but has no effect on TNFα shedding at concentrations up to 100 μM. A Phase I studies in advanced cancer patients revealed grade 1 myalgia/ arthralgia in 7 of 28 patients and grade 2 in 3 of 28 patients.[66] While this data suggest that inhibition of TACE itself may not mediate joint effects, inhibition of related metalloproteinases can not be ruled out.

The issue of MMP inhibitor efficacy currently remains largely unresolved. Results from three Phase III trials have been reported for marimastat and are discussed in detail in Section XV.A.1. Marimastat did not demonstrate a significant improvement in survival among pancreatic cancer patients when given as a single agent or in combination with gemcitabine.[67] This is in contrast to a study in advance gastric cancer where the compound produced a strong trend toward increased survival among all patients and a statistically significant increase in survival among patients who presented without evidence of metastasis.[68] Both trials indicate that MMP inhibitors are likely to be more effective in earlier stage disease, thereby providing critical information regarding appropriate patient populations for clinical trials with Abbott's MMP inhibitor. Results from additional pivotal trials with marimastat and prinomastat are expected by mid-year. Analysis of these results must take into account the inability to explore higher doses of these agents due to unacceptable joint effects.

D. Abbott Approach

Given the substantial evidence implicating both gelatinase A and gelatinase B as contributors to tumor progression (Section V.B.2), these enzymes were chosen as the primary biochemical targets. In the absence of definitive evidence regarding the cause of the MMP inhibitor-induce joint toxicity, emphasis was placed on compounds which spare inhibition of fibroblast collagenase and related metalloproteinases (gauged through TACE inhibition). Several facts support this decision. First, doses of primonastat which produce plasma concentrations significantly higher than its IC_{50} for fibroblast collagenase are associated with joint toxicity.[61] Significantly, doses of prinomastat which *do not* produce joint effects give rise to plasma

concentrations substantially higher than its IC_{50} for the gelatinases, even after correcting for plasma protein binding. Furthermore, joint effects were not observed following chronic dosing of the highly gelatinase selective inhibitor, BAY 12-9566. This compound is only modestly potent versus gelatinase A (120 nM), but possesses no fibroblast collagenase or TACE inhibitory activity (Section XV.A.4). Clearly, these data go further in acquitting gelatinase inhibition than implicating fibroblast collagenase/ TACE inhibition in mediating joint toxicty. Yet they are supportive of the Project Team's goal of identifying highly gelatinase selective MMP inhibitors as a means of gaining an advantage over competitor's compounds.

VI. Targeted Product Profile

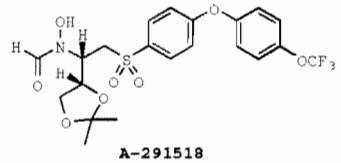
Abbott's MMP inhibitor must provide clinically significant advantages in efficacy (an increase in survival or time to progression) or tolerability (minimal joint effects) relative to the competitors. Chronic administration is anticipated so oral activity and an acceptable safety profile are critical attributes for a viable product. For the reasons outlined in Section V, compounds with high intrinsic potency versus gelatinase A & B (IC_{50} < 10 nM), and which lack activity versus fibroblast collagenase and sheddases like TACE, may be efficacious without producing joint toxicity. Results from cancer animal models suggest that sustained exposure to plasma concentrations exceeding the gelatinase A & B IC_{50} values are necessary for optimal antitumor effects.[69] Given this, and the need to avoid plasma concentrations necessary to inhibit proteinases involved in tissue remodeling, an extended *in vivo* half-life (> 6 hours) is important. A finished cost of goods that is consistent with at least an 80% standard manufacturing margin is another targeted product attribute.

VII. Physical Chemical Information

A. Physiochemical Properties

1. Structure, Solid State Properties and Lipophilicity

A-291518.0 (referred to below as A-291518) is a non-hygroscopic white crystalline powder with a melting point of approximately 130°C. Thermogravimetric analysis shows no evidence of desolvation and amorphous material recrystallizes at 85°C on heating. The logarithm of the distribution coefficient between octanol and pH 7.4 buffer is 3.43.



A-291518

Molecular formula: $C_{21}H_{22}F_3NO_8S$

Molecular weight: 505.46 g/mole

IUPAC Name

[S-(R*,R*)]-*N*-[1-(2,2-dimethyl-1,3-dioxol-4-yl)-2-[[4-[4-(trifluoromethoxy)-phenoxy]phenyl]sulfonyl]ethyl]-*N*-hydroxyformamide

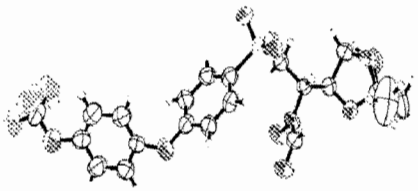


Figure 2. Structural formula, molecular formula, IUPAC name and ORTEP plot (carbon - black, oxygen - red, nitrogen - blue, sulfur - yellow, hydrogen and fluorine - green) for A-291518.

2. pK_a and Solubility

The predicted value for the pK_a of A-291518 is 7.94. This was confirmed experimentally by analysis of the pH-solubility profile. The solubility of the compound was assessed in a variety of media (Table 5). It has very low aqueous solubility (5 µg/ mL) in the pH range normally accessible in the gastrointestinal tract (pH = 1 to 7.4.) The solubility increases to over 1 mg/ mL under basic conditions (pH > 10). At alkaline pH the compound exhibits surface activity which may present the potential for hemolysis if an intravenous formulation of a salt is developed.

Table 5. Solubility of A-291518 in various media at 25°C.

Solvent	Solubility; mg/ ml (± SD)
aqueous buffer, pH 1	0.005 (0.0001)
aqueous buffer, pH 6.8	0.006 (0.0002)
aqueous buffer, pH 10.4	1.34 (0.02)
Capmul MCM[a]	34.1 (0.3)
Ethanol	54.1 (0.5)
Oleic Acid	2.2 (0.1)
Oleic acid+5% Ethanol	6.9 (0.1)
PEG 400	70.6 (3.4)
100 mM Sodium Dodecyl Sulphate (aqueous)	4.9 (0.3)

a. Medium chain monoglycerides, Abitec Corporation.

B. Preformulation

1. Stability

A-291518 is stable in the solid state upon exposure to 40°C/ 75% relative humidity for 6 months. In solutions at pH 7.4 and 13, the compound slowly degrades with half-lives of 29 and 60 days respectively. Degradation of A-291518 is accelerated at pH 1 with a half-life of 17 minutes. This would normally require an enteric coated formulation, however the low solubility of the free acid may minimize the extent of degradation. Exposure of solid or solutions to visible and UV light does not significantly affect the degradation kinetics.

2. Phase I Formulation/ Commercial Formulation

The solubility exhibited by A-291518 is probably not sufficient to allow an aqueous solution dosage form for Phase I trials. The crystalline solid tends to form large clumps therefore excipients may be required to improve handling. Food assists in the absorbtion of A-291518 in dogs (Section XII.A). Thus, administration of the compound as a solid in capsules with excipients and dosed with food, should be an acceptable dosing regimen for Phase I studies.

One advantage of A-291518 over ABT-770 is its stability at elevated pH. This allows the possibility for preparing a retrohydroxamate salt which could lead to improved solubility and faster dissolution of the compound. A soluble salt may diminish the effect of food on bioavailability. In the absence of an appropriate salt, formulation with wetting agents or as a suspension in blends of lipid components may provide an avenue for an oral dosage form with reduced food effect.

VIII. Patent Status

A patent application entitled "Reverse Hydroxamate Inhibitors of Matrix Metalloproteinases" was filed in the United States Patent and Trademark Office in August, 1997 (Abbott case 6162.US.01). A continuation-in-part (CIP) application (Abbott case 6162.US.02) and the corresponding PCT application (Abbott case 6162.PC.01) were filed in August, 1998. Separate Notices of Allowances for both U.S. patent applications have been received. The PCT application will be nationalized in various countries in March 2000.

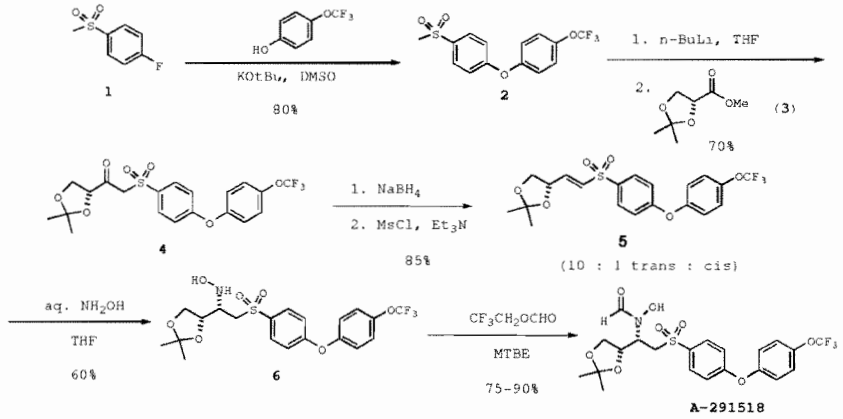
Prior to the publication of the PCT application in February 1999, a second application (Abbott case 6162.US.P1) was filed specifically claiming A-291518. The Project Team intends to abandon

this application in lieu of an additional CIP (Abbott case 6162.US.P3) which was filed in January of 2000. This strategy will provide broader substance of matter protection than found in Abbott case 6162.US.02. To obtain coverage outside the U.S., a separate PCT application (Abbott case 6162.PC.03) which specifically claims A-291518 was filed in January of 2000.

IX. Synthetic Chemistry

Project Team chemists have developed a 6-step synthesis of enantiomerically pure A-291518 from commercial starting materials which has allowed for the preparation of multigram quantities. The route has been adopted by the process chemistry team and will be used to prepare multi-kilogram quantities of bulk drug for pre-clinical and Phase I clinical evaluation. Reaction of *p*-fluorobenzene methylsulfone with *p*-trifluoromethoxyphenol under basic conditions gives the biaryloxysulfone 2 in high yield (Scheme 1). Deprotonation of sulfone 2 with *n*-BuLi, followed by reaction with methyl (R) 2,2-dimethyl-1,3-dioxolane-4-carboxylate (3) gives the ketone 4 in 70% crystallized yield. Reduction and subsequent elimination via the corresponding mesylate affords the olefin 5 in high yield. Michael addition of hydroxylamine to the olefin affords the adduct 6 as a 4 to 1 mixture of diastereomers. The desired syn diastereomer is separated via recrystallization and is then formylated to give A-291518 in 75-90% yield and greater than 99% enantiomeric purity. The overall yield for A-291518 is 25%. A preliminary cost of goods analysis suggests an estimated cost for A-291518 of approximately $4,800/ kg, based on a projected annual demand of 10,000 kg.

Scheme 1. Synthetic Route to A-291518.



X. *In Vitro* Characterization

A-291518 displays potent inhibition of gelatinase A and greater than 10,000-fold selectivity versus fibroblast collagenase. In contrast to ABT-770, A-291518 is also a potent inhibitor of gelatinase B. A-291518 exhibits only modest inhibition of TACE (IC_{50} = 340 nM) and, in contrast to marimastat, does not inhibit cellular TNFα release. The degree of selectivity for inhibition of the gelatinases relative to fibroblast collagenase is substantially higher for A-291518 than for marimastat or prinomastat.

A. Inhibition of Matrix Metalloproteinases

A-291518 is a potent inhibitor of gelatinase A and possesses a high degree of selectivity relative to fibroblast collagenase (Table 6). It is 200-fold more potent versus gelatinase B than ABT-770. A-291518 is also a potent inhibitor of neutrophil collagenase, stromelysin and collagenase-3, but

exhibits weak activity against MT1-MMP and does not inhibit matrilysin. Marimastat is a broad spectrum MMP inhibitor, with similar potency against all of the MMPs tested. Prinomastat is a very potent inhibitor of the gelatinases, but displays substantial activity as an inhibitor of the other MMPs. Its fibroblast collagenase to gelatinase A potency ratio (120) is 100-fold less than A-291518.

Table 6. Inhibition of Matrix Metalloproteinases.[a]

Compound	MMP-2	MMP-9	MMP-1	(MMP-1/ MMP-2)	MMP-3	MMP-7	MMP-8	MMP -13	MMP -14
				IC_{50} (nM)					
A-291518	0.78	0.50	8,900	(11,400)	12	11,000	5.0	3.3	180
ABT-770	3.7	120	4,600	(1,240)	42	>10,000	7.8	45	580
marimastat	0.41	0.79	0.78	(2)	14	4.1	0.47	1.2	1.9
prinomastat	0.048	0.048	5.7	(120)	3.5	72	0.54	0.20	1.1

a. MMP-1: fibroblast collagenase; MMP-2: gelatinase A; MMP-3: stromelysin; MMP-7: matrilysin; MMP-8: neutrophil collagenase; MMP-9: gelatinase B; MMP-13: collagenase-3; MMP-14: MT1-MMP.

B. Inhibition of Other Metalloproteinases

Since compounds which have a metal chelating moiety may inhibit other metalloproteinases than those of the MMP family, four other enzymes, listed in Table 7, have been used to determine the specificity of inhibition by the compounds discussed above. A-291518 does not inhibit neprilysin, leucine aminopeptidase or thermolysin and possesses marginal activity versus TACE. In contrast, marimastat is a potent inhibitor of TACE, and exhibits more inhibitory activity than the A-291518 when tested against the other unrelated metalloproteinases. Prinomastat is a potent inhibitor of TACE exhibiting an IC_{50} value of 7.9 nM.

Table 7. Inhibition of Cellular TNFα Release, TACE and other Metalloproteinases.

Compound	TML[a]	NEP[b]	LAP[c]	TACE[d]	Cellular TNFα release[e]
	IC_{50}(nM)				IC_{50}(μM)
A-291518	>100,000	>100,000	>100,000	340	>50
ABT-770	>100,000	>100,000	>100,000	18,000	>50
marimastat	1,100	500	4,600	1.8	2.1
prinomastat	>100,000	>100,000	>100,000	7.9	8.6

a. TML, thermolysin (*B. thermoproteolyticus*); b. NEP, rat neprilysin, EC# 3.4.24.11; c. LAP, leucine aminopeptidase (porcine kidney); d. TACE, recombinant human TNFα-converting enzyme; e. Release of TNFα from LPS-stimulated THP-1 cells.

While the physiological ramifications of TACE inhibition have not been fully characterized, it serves as a representative of the ADAM class of metalloproteinases. Among other activities, these enzymes have been shown to be involved in the proteolytic release of membrane bound proteins from the surface of cells (termed "sheddase" activity). In contrast to marimastat and prinomastat, the inhibitory effect of A-291518 on TACE does not translate into inhibition of cellular TNFα release. That is, marimastat blocks LPS-stimulated TNFα release from THP-1 cells with an IC_{50} value of 2.1 μM whereas A-291518 had no effect in this assay at concentrations exceeding 50 μM.

C. Plasma Protein Binding

1. Centrifugation Studies

A-291518 was evaluated for protein binding by direct measurement in human and mouse plasma. It exhibits high protein binding, more so in human plasma than in mouse plasma (Table 8). The determination of protein binding performed at Abbott for prinomastat is consistent with reports from Agouron scientists.[69]

Table 8. Plasma Protein Binding.

Compound	% plasma bound ± SEM mouse	human
A-291518	94.4 ± 0.24	99.2 ± 0.04
ABT-770	95.0 ± 0.48	96.7 ± 0.28
prinomastat	81.6 ± 2.1	78.2 ± 1.5

2. Enzyme Inhibition Determined in the Presence of Human Plasma

In order to assess the functional consequence of high protein binding, an assay was devised in which compounds were evaluated as inhibitors of a recombinant truncated form of gelatinase A in the presence of 80% normal human plasma. In order to overcome endogenous inhibitors, and because of the background fluorescence of the plasma, it was necessary to add 20-fold more enzyme to the plasma than when carrying out the same assay in buffer. It was also necessary to use a substrate blocked at the amino terminus and carboxy terminus to prevent degradation of the substrate by plasma exopeptidases. In buffer assays, the compounds inhibit the truncated enzyme at 2-3 fold higher concentrations than in the screening assay with native enzyme. Some of this shift, particularly evident with prinomastat, may be due to higher enzyme concentrations used in the assays with truncated gelatinase A.

The data in Table 9 demonstrate that A-291518 can inhibit gelatinase A at concentrations achievable in plasma. The magnitude of the shift in potency for A-291518 is in the range expected for compounds which are substantially (> 90%) bound to plasma proteins. Marimastat and prinomastat maintain nanomolar potency when assayed in the presence of plasma.

Table 9. Shift in Gelatinase A Potency in the Presence of Human Plasma.

Compound	Gelatinase A Inhibition (IC_{50}, nM) truncated gelatinase A in 80% plasma	truncated gelatinase A in buffer	fold-shift in potency	native gelatinase A in buffer
A-291518	100	1.9	53	0.78
ABT-770	110	11	10	3.7
marimastat	6.0	2.0	3	0.41
prinomastat	5.4	1.5	3.6	0.048

XI. *In Vivo* Pharmacology

A-291518 demonstrates antitumor activity when administered orally as monotherapy in a murine syngeneic tumor growth model (B16 melanoma). Inhibition of tumor growth is dose dependent and correlates with concentration of drug in blood. The potency of A-291518 in the B16 model is comparable to ABT-770 at a blood exposure (AUC) approximately 10-fold less than ABT-770. A-291518 is also efficacious in human xenograft models. In a pancreatic carcinoma model (MiaPaCa) and in a fibrosarcoma model (HT1080) the compound exhibits activity comparable to ABT-770 and prinomastat. A-291518 also demonstrates efficacy in an orthotopic human colon cancer model. When given with cytotoxic agents, combination therapy with A-291518 results in tumor growth delays greater than with either agent given alone. In addition, A-291518 is effective in blocking b-FGF and VEGF-induced blood vessel formation in the mouse cornea model of angiogenesis.

A. Tumor Models in Rodents

The principle endpoint for tumor models in rodents has been growth of primary tumors. Tumor size is calculated using the formula [L x W^2/ 2], which is a valid estimate of mass assuming unit density.[70] Mean and median time to a particular tumor size are calculated for

drug-treated and untreated groups. Efficacy of drug therapy against solid tumors can be represented as the growth delay or percent increase in life span (%ILS) following tumor implantation. This value is usually standardized to the growth rate of the untreated tumor (t_t - t_c)/ t_c.[71] In addition, inhibition of solid tumor growth on any given day can be represented as mean mass of the test drug group over the mean mass of the untreated group (T/ C) or as inhibition of control (1-T/ C).[72]

1. Murine Syngeneic

B16 melanoma. Tumors derived from the B16 cell line (murine melanoma) have served as a primary model for the evaluation of MMP inhibitors for growth inhibition. The B16 cells, when dispersed in Matrigel and implanted subcutaneously in the flank of mice, grow rapidly as tumors, reaching 1 gram in 13-14 days. B16 cells grown in culture, as well as tumor tissue recovered from the host, contain relatively high levels of mRNA for gelatinases A and B.[73] The syngeneic tumors are sensitive to conventional cytotoxic agents such as cyclophosphamide and paclitaxel and thus provide an appropriate model for evaluating MMP inhibitors in combination therapy.

Figure 3 (left) shows the results from one study with A-291518 in the B16 flank tumor model. Oral administration of the compound, starting 7 days after tumor cell implantation, resulted in dose-dependent inhibition of tumor growth. Statistically significant inhibition was observed at oral doses of 3 mg/ kg, bid, and higher.

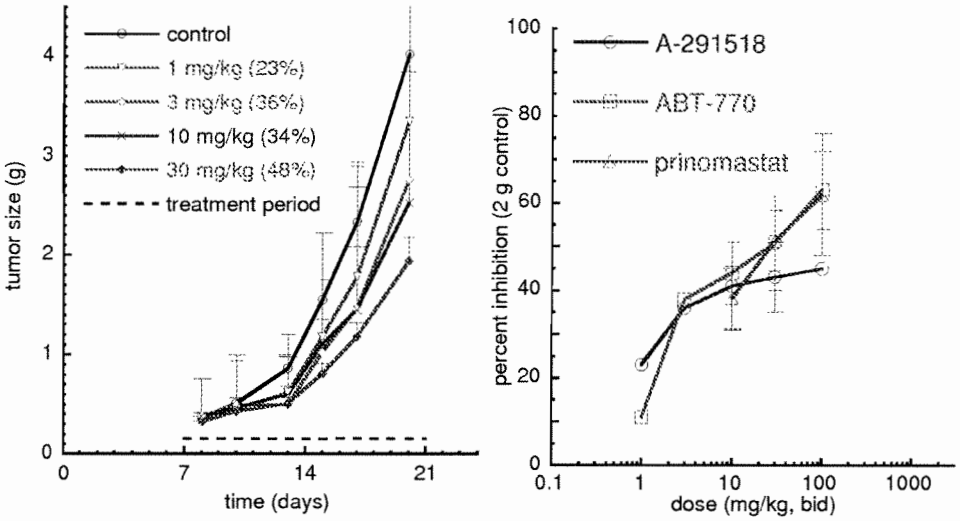


Figure 3. Growth inhibition of murine melanoma. *Left* Effects of A-291518 on the growth of B16 melanoma cells implanted subcutaneously in the flank of mice. Compound or vehicle was administered orally twice daily on days 7 - 21. Tumor volumes are expressed as mean ± SEM, n = 10 per group. Significant differences ($p<0.05$ vs control) in mean tumor volume were observed for the 3, 10 and 30 mg/ kg dose groups by day 13 for A-291518. Percent inhibition of control (2 g) is indicated in parenthesis in the legend for each group. *Right* Inhibition as a function of dose. Values are expressed as the mean ± sd percent inhibition of control at 2 g from multiple studies (ABT-770: 10, and 100 mg/ kg, n=2; 30 mg/ kg, n=3; prinomastat: 10 and 100 mg/ kg, n=2; 30 mg/ kg, n=5; A-291518: 10 mg/ kg, n=2, 30 mg/ kg, n=3).

A comparison of the effects on inhibition of tumor growth with A-291518 from the above and other studies with the historical data for ABT-770 and prinomastat is shown in the Figure 3 (right). Inhibition of tumor growth was comparable among the Abbott and reference inhibitors.

Combination studies. It is likely that MMP inhibitors will be used in combination with conventional cytotoxic therapies in the treatment of cancer. To validate this approach

experimentally, studies with paclitaxel, in combination with A-291518 were undertaken with the B16 model. Sub-optimal doses of the cytotoxic agents were used in order to reveal possible additive or synergistic effects.

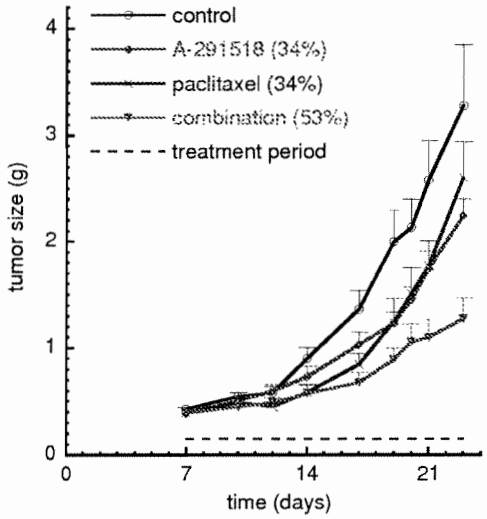


Figure 4. Effects of A-291518 and paclitaxel on the growth of B16 melanoma cells implanted subcutaneously in the flank of mice. A-291518 was administered orally (30 mg/ kg) twice daily on day 7 - 21. Paclitaxel (12.5 mg/ kg) was administered ip once a day for 3 days starting on day 7, followed by once every 3 days through day 21. Results are expressed as mean + SEM, n = 10 per group. Mean tumor volume of the combination treatment groups differed significantly ($p<0.05$) from the mono-therapy groups starting on day 21 for A-291518. Percent inhibition of control (2 g) is indicated in parenthesis in the legend for each group.

As illustrated in Figure 4, administration of A-291518 in combination with paclitaxel resulted in greater inhibition of tumor growth than that achieved with either agent alone. No symptoms of overt toxicity such as weight loss were observed throughout. These results suggest that combination therapy with an MMP inhibitors can improve the effectiveness of conventional cytotoxic therapies.

2. Human Xenograft

HT1080 fibrosarcoma. The antitumor efficacy of MMP inhibitors has been evaluated against tumors derived from the HT1080 human fibrosarcoma cell line. These tumors grow to 2.5 grams in SCID Beige mice in 14-16 days with a doubling time of approximately 3 days when implanted subcutaneously from cell culture. Cells grown in culture, as well as tumor tissue recovered from the host, contain relatively high levels of mRNA for human gelatinase A. The HT1080 cell line is also known to express MMP-9, MT1-MMP, TIMPs, and VEGF.[74]

Results from a representative study with A-291518 in the HT1080 model are shown in Figure 5 (left). A-291518 produced dose-dependent inhibition of tumor growth. The extent of inhibition achieved with A-291518 in the HT1080 model was comparable to that of ABT-770 and prinomastat (Figure 5, right).

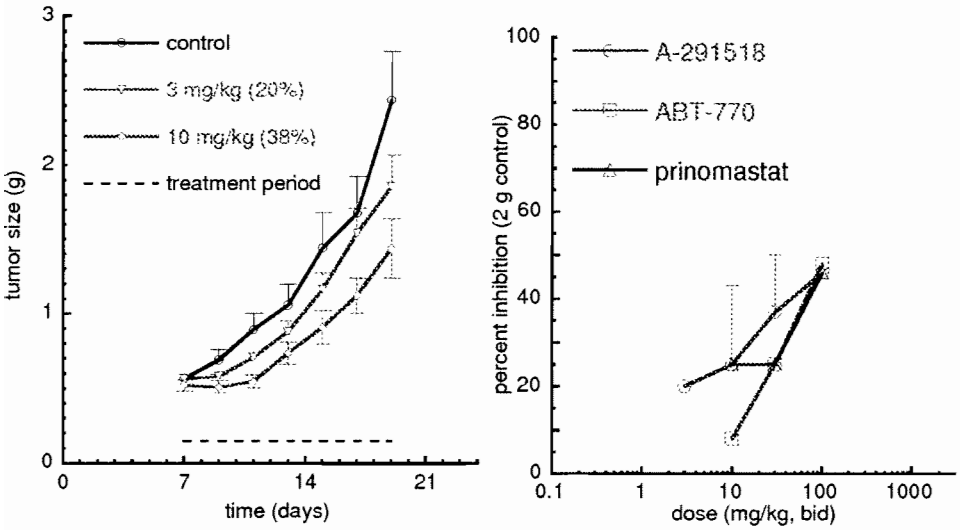


Figure 5. Growth inhibition of human fibrosarcoma. *Left* Effects of A-291518 on the growth of HT1080 cells implanted subcutaneously in the flank of SCID Beige mice. Compound or vehicle was administered orally twice daily on days 7 - 21. Tumor volumes are expressed as mean ± SEM, n = 10 per group. Significant differences ($p<0.05$ vs control) in mean tumor volume were observed for the 10 mg/ kg dose group of A-291518 by day 9. Percent inhibition of control (2 g) is indicated in parenthesis in the legend for each group. *Right* Inhibition as a function of dose. Values are expressed as the mean ± sd percent inhibition of control at 2 g. In cases of multiple studies (A-291518, 10 and 30 mg/ kg, n=2) percent inhibition values were pooled.

MDA-435 breast carcinoma. The antitumor efficacy of A-291518 was evaluated against tumors derived from the MDA-435 human breast carcinoma cell line. These tumors grow orthotopically in the breast fat pad when implanted as breis prepared from existing tumors.

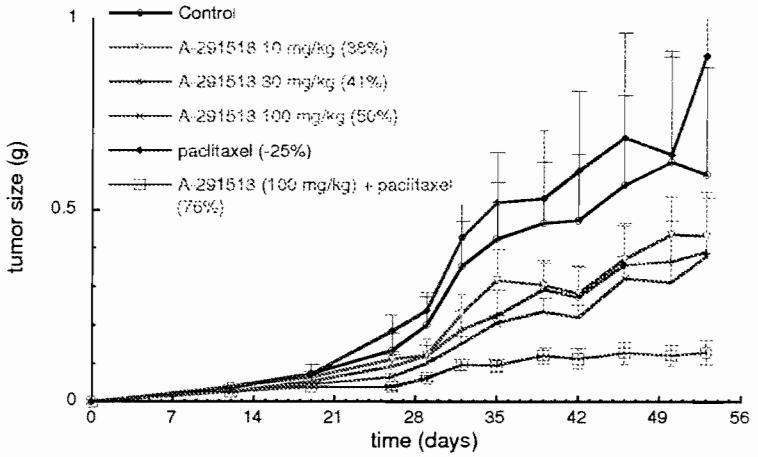


Figure 6. Effects of A-291518 on the growth of MDA-435 orthotopic xenografts in nude mice. A-291518 was administered orally at 10, 30, and 100 mg/ kg, bid. Paclitaxel (10 mg/ kg, ip) was administered on days 1, 5 and 9. Results are expressed as mean ± SEM. Percent inhibition of control (2 g) is indicated in parenthesis in the legend for each group.

A-291518 inhibited tumor growth in the breast orthotopic model, although the dose-response to the agent was highly compressed (Figure 6). Perhaps the most significant observation from this study is the profound inhibitory effect of the combination therapy relative to the control and monotherapy groups.

MiaPaCa pancreatic carcinoma. MiaPaCa is a human pancreatic carcinoma-derived cell line. Known mutations include oncogenic K-ras, p53 inactivation, and inactivation of the TGFβ type II receptor.[75] These xenografts grow rapidly in nude mice, reaching 1 gram in 15-21 days and are sensitive to cyclophosphamide and gemcitabine.

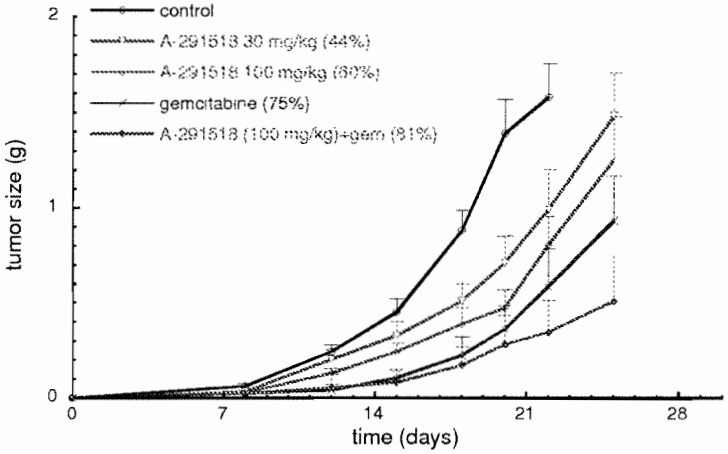


Figure 7. Effects of A-291518 as mono-therapy or in combination with gemcitabine on the growth of MiaPaCa xenografts in nude mice. A-291518 was administered orally at 30, and 100 mg/ kg, bid. Gemcitabine (10 mg/ kg ip) was administered on days 1, 4, 7 and 10. Results are expressed as mean ± SEM. Percent inhibition of control (1 g) is indicated in parenthesis in the legend for each group.

Results from a study with the MiaPaCa model are shown in Figure 7. A-291518 inhibited growth of the pancreatic carcinoma xenograft in a dose-dependent manner. The inhibition achieved in these studies was marginally higher than that previously obtained with ABT-770 (29% at 30 mg/ kg bid, Table 11). However, conclusions about potency differences will require definitive head-to-head studies. As was seen with the previous tumor models, combination therapy, in this case with gemcitabine and A-291518, was more effective than mono-therapies of either agent.

Co-3 colon adenocarcinoma. Tumor growth, survival and antimetastatic effects were measured against tumors derived from orthotopic implantation of Co-3 human tumor xenografts in nude mice in studies conducted by AntiCancer Inc., San Diego.[76] Co-3 tumors were derived from the metastatic lung lesion of a well-differentiated colon adenocarcinoma of a female patient.[77] Orthotopically grown tumors exhibit gelatinase B activity as determined by zymography. The growth and metastatic spread of these tumors was inhibited by a selective gelatinase inhibitor from Celltech Ltd., CT1746; this compound also prolonged the mean survival time of the tumor-bearing animals.[78]

As shown in Figure 8 (left), A-291518 and ABT-770 both inhibited the growth of Co-3 tumors. Mean tumor volumes for the A-291518 (20 mg/ kg, bid, po) and 5-FU dose groups were significantly different from the vehicle control group at Day 47 (87 and 98% inhibition, respectively). The lack of distinction between the two doses of A-291518 may be due to variability in the tumor take rates in this model.

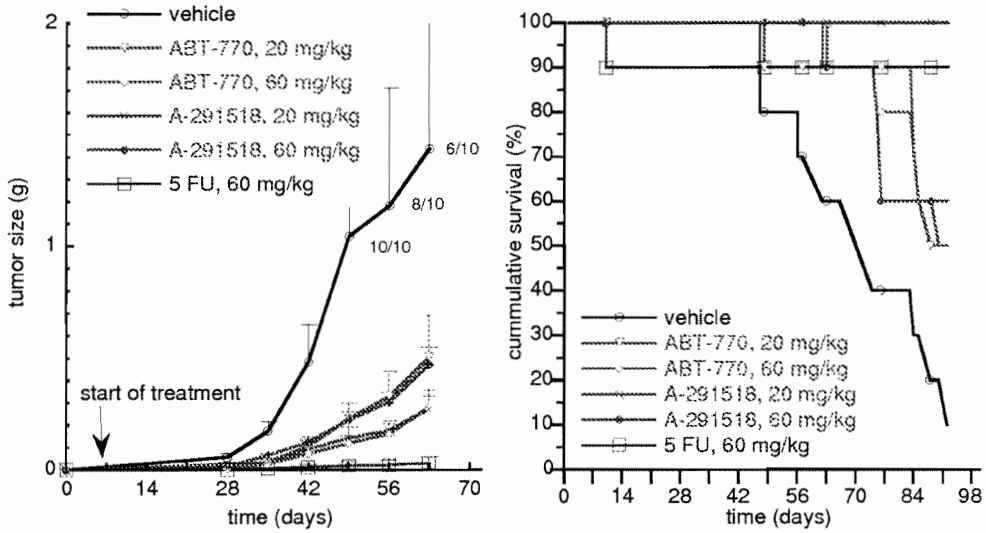


Figure 8. Effects of A-291518 and ABT-770 on Co-3 tumors. *Left* Inhibition of tumor growth. Tumor fragments (1 cubic millimeter, 3 per mouse) were implanted surgically onto the ascending colons of male Crl-nu athymic nude mice. Compound or vehicle was administered orally twice daily on day 5 post surgery. Tumor sizes are expressed as mean ± SEM, n = 6-10 per group. Significant differences ($p<0.05$ vs control) in the mean tumor volumes for the treated groups compared to the vehicle group were observed at Day 47 when the first control animal died. *Right* Kaplan-Meier Plot of Survival vs. Time for A-291518 and ABT-770 treated groups.

A-291518 also prolonged survival of tumor-bearing animals as illustrated by the Kaplan-Meier plot (Figure 8, right). There was a 100% and a 60% survival rate at 20 and 60 mg/ kg, bid, respectively, for A-291518 when 90% of the vehicle controls died (Table 10). For ABT-770 at 20 and 60 mg/ kg, bid, a 50% survival rate was observed.

Table 10. Effect of A-291518 on Survival Rate.

Group	Survival Rate (%)	P-value[a]
Vehicle	10	---
A-291518, 20 mg/ kg	100	<0.001
A-291518, 60 mg/ kg	60	0.057
ABT-770, 20 mg/ kg	50	0.141
ABT-770, 60 mg/ kg	50	0.141
5-FU, 60 mg/ kg	90	0.001

a. P-values were determined by the Fisher-exact test. Survival in each treated group (n=10 animals at day 0) was compared with the vehicle control at day 92 when 90% of the controls died and the experiment was terminated.

The incidences of metastasis and local invasion were evaluated in distant organs and local tissues by microscopic examination after hematoxylin and eosin staining at necropsy. Local invasion of the primary tumor into the abdominal wall, cecum and ileum was observed in 7 of 10 animals of the vehicle control group. Only in the ileum was the incidence of local invasion significantly less ($p< 0.02$) for groups treated with 20 mg/ kg, bid, po A-291518 and 60 mg/ kg, ip, 5-FU as compared to the vehicle control. There were no differences between any of the other treatment groups and the vehicle control. Metastatic lesions for the vehicle treated group were only observed in the mesenteric lymphnodes of 3 of 10 animals and no metastases were observed in the liver or lungs. There were no differences between the incidence of metastasis between the vehicle controls and any of the drug treated groups.

Exhibit A
Part 3

3. Blood Levels in Efficacy Models

In an effort to relate efficacy to drug blood level, the presence of parent drug was assayed in blood samples obtained over a 12 hour period after the last dose at the end of the efficacy studies. Concentration was determined by HPLC. The results of this analysis for the B16 studies with A-291518 and ABT-770 are shown in Figure 9.

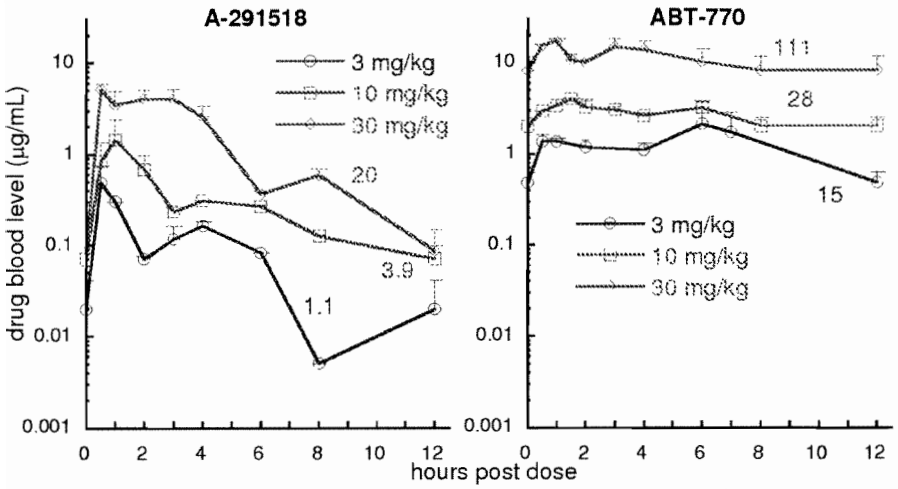


Figure 9. Blood levels of A-291518 (*left*) and ABT-770 (*right*) on Day 21 (14 days of dosing). Results are expressed as mean ± SD n = 2. AUC (µg•hr/ mL) values are shown for each dose.

Inspection of blood levels of A-291518 reveals a fairly large ratio (>10 fold) between C_{max} and C_{min}. Thus in contrast to ABT-770, A-291518 did not reach a steady-state concentration in the efficacy studies. It is therefore difficult to estimate with certainty the minimal concentration necessary to achieve a biological significant effect. Using C_{max} as a guide, significant efficacy was achieved with A-291518 at blood concentrations below 0.5 µg/ mL (Figure 9, left).

The blood level studies can also be used to examine the relationship between total exposure and efficacy. The total exposure, expressed as area under the curve (AUC) values, achieved in the B16 studies ranged from 1 to 20 µg•h/ mL for A-291518 and was approximately proportional to dose. An estimate based upon the AUC value at a dose necessary to achieve at least 30% inhibition (3 mg/ kg) indicates a 12 h exposure in the range of 1 µg•h/ ml for A-291518 (Figure 10). By comparison, the 12 h AUC value for ABT-770 at its minimum efficacious dose (also 3 mg/ kg) was approximately 15 µg•h/ mL. These results serve as the bases for estimating the potential therapeutic window of A-291518 (Section XIII.G).

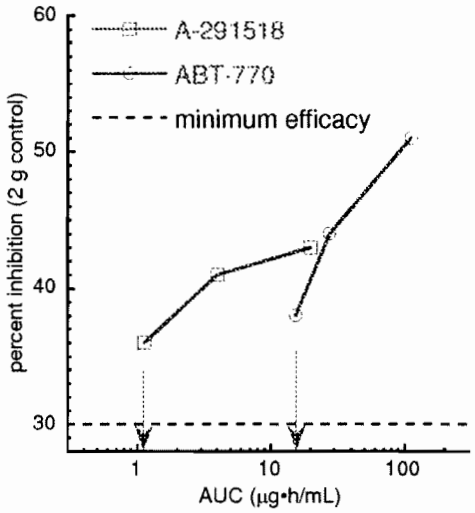


Figure 10. Relationship between AUC and efficacy (percent inhibition) in the B16 tumor growth model.

B. Angiogenesis

Formation of new blood vessels is critical for the rapid growth of solid tumors. Tumor cells and surrounding stroma stimulate vascularization by secreting angiogenic factors that induce endothelial cell migration, proliferation and capillary formation. MMPs, due largely to their role in extracellular matrix degradation that is necessary for vessel growth, have been implicated in the process of neovascularization associated with tumor angiogenesis.[20, 21, 23] Experimentally, implantation into a mouse cornea of a controlled polymer embedded with basic-fibroblast growth factor (b-FGF) or vascular endothelial growth factor (VEGF) results in local neovascularization of the cornea that can be quantified by image analysis.

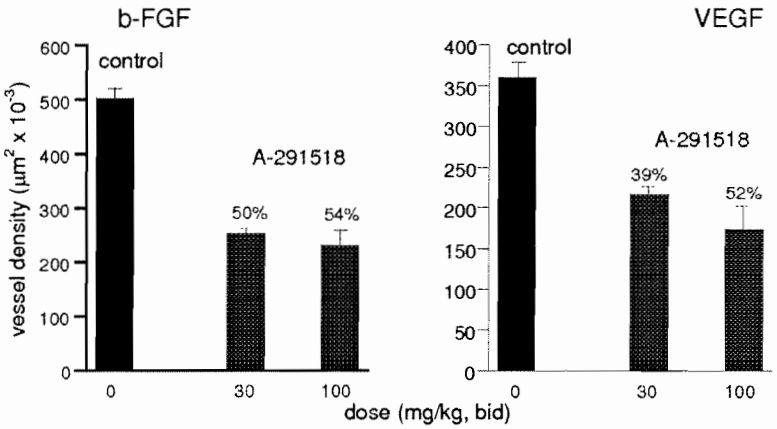


Figure 11. Inhibition of bFGF- (*left*) and VEGF- (*right*) induced angiogenesis. On study Day 0, corneas of CF-1 mice were implanted with a pellet containing either 30 ng of bFGF or (contralateral) 150 ng VEGF mixed with sucralfate (to stabilize the growth factor) and hydron (to slow the release of the growth factor). Therapy was initiated the evening of Day 0, after recovery from anesthesia, and continued until Day 5. Corneal neovascularization was assessed on the day after therapy ended. Each of the four treatment groups differed significantly from control ($p < 0.01$).

Results from a study with A-291518 in the cornea model are illustrated in Figure 11. A-291518 had a significant effect on both b-FGF and VEGF-induced responses. Similar effects have been observed previously with prinomastat and ABT-770 (Table 11).

C. Summary of *In Vivo* Efficacy

The inhibitory effects of A-291518 in tumor growth and angiogenesis models are summarized in Table 11. The effectiveness of A-291518 and of other reference MMP inhibitors in a relatively broad range of experimental cancer models provides evidence of the potential clinical utility of this class of therapy and supports further development of the Abbott MMP inhibitor.

Table 11. Summary of *In Vivo* Efficacy in Tumor and Angiogenesis Models.

	Tumor	prinomastat	ABT-770	A-291518
B16	mouse melanoma	51 ± 7 (n=5)	51 ± 9 (n=3)	44 ± 6 (n=4)
HT1080	human fibrosarcoma	25	25 ± 1 (n=3)	37 ± 13 (n=2)
MDA-435	human orthotopic breast carcinoma	*nt*	60	38
MiaPaCa	human pancreatic carcinoma	40	29	54 ± 25 (n=3)
Co-3	human orthotopic colon carcinoma	*nt*	82	88
mouse cornea angiogenesis				
	b-FGF	38 ± 19	53 ± 19	29 ± 14
	VEGF	75 (n=2)	23 ± 4 (n=2)	43 ± 9 (n=3)

Values for efficacy in the tumor models are expressed as percent inhibition of control at 0.5 g (MDA-435), 1 g (MiaPaCa, Co3), or 2 g (B16, HT1080). In cases of multiple studies percent inhibition values were pooled and expressed as the mean ± sd. Compounds were administered po at a dose of 30 mg/ kg, bid with the exception of Co3 (dose of 60 mg/ kg, bid). nt: not tested.

XII. Pharmacokinetics and Metabolism

A-291518 exhibits oral bioavailabilities between and 68 and 93% in rats, dogs and monkeys. The oral bioavailability of non-formulated capsules of A-291518 is enhanced in the presence of food. Metabolites analogous to those formed by ABT-770 are produced following multiple oral doses in rats and monkeys, the absolute and relative amounts being gender and species dependent. However, the concentration of metabolites in tissues of chronically treated rats and monkeys is far less for A-291518 than for ABT-770. Exposure to A-291518 in multiple dose studies varies with time suggesting the potential for induction of metabolizing enzymes.

A. Single Dose Pharmacokinetics

The pharmacokinetics of A-291518 were evaluated in Sprague-Dawley rat, beagle dog and cynomolgus monkey. Data obtained from these studies was compared to that obtained in similar studies with ABT-770. The pharmacokinetic behavior of A-291518 following a 3 mg/ kg intravenous dose in rat, dog and monkey was characterized by a variable terminal elimination half-life with values ranging from 3.2 (rat) to 12.9 (monkey) hours (Table 12). Plasma clearance values were very low, decreasing from rat (0.13 L/ hr•kg) > dog (0.09 L/ hr•kg) > monkey (0.076 L/ hr•kg). A-291518 was characterized by volume of distribution values (V_β) lower than those obtained for ABT-770. A-291518 plasma clearance values were very similar to those obtained with ABT-770 in all three species.

Table 12. Pharmacokinetics of ABT-770 and A-291518 after a 3 mg/ kg Single IV or Oral (solution) Dose in Rat, Monkey and Dog.

	IV Dose (3 mg/kg)					Oral Dose (3 mg/kg)					
Species	$t_{1/2}$	V_c	V_β	AUC	Cl_p	C_{max}	T_{max}	$t_{1/2}$	AUC	F	n
						ABT-770					
Rat	7.7	1.2	1.3	25.63	0.12	1.33	2.5	8.2	23.84	93.0	4
Dog	8.7	0.9	1.4	28.06	0.11	1.87	0.9	8.4	22.82	83.0	6[a]
Monkey	21.0	1.2	2.1	44.97	0.068	1.55	2.7	23.0	42.53	95.0	6[a]
						A-291518					
Rat	3.2	0.6	0.6	25.07	0.13	2.12	3.0	5.0	22.01	87.8	3
Dog	5.0	0.3	0.7	35.62	0.09	4.88	0.6	6.0	33.52	93.2	6[a]
Monkey	12.9	0.3	1.4	39.35	0.076	1.25	7.6	11.7	26.67	67.6	5[a]

Units: $t_{1/2}$ (hr); V_c (l/ kg); V_β (l/ kg); AUC (µg•hr/ ml); CL_p (l/ hr•kg); Cmax (µg/ ml); T_{max} (hr); F(%).

a. crossover study: absolute bioavailability.

A-291518 was slowly absorbed in rat with peak plasma concentrations (C_{max} = 2.12 µg/ ml) noted ~3 hours after oral dosing. A-291518 was characterized by a plasma elimination half-life of 5 hours with 87.8% bioavailability in the rat. These peak plasma concentrations were slightly higher than obtained for ABT-770, with slightly faster plasma elimination half-lives and marginally lower bioavailability values (Table 12). A-291518 was characterized by high peak plasma concentrations (C_{max} = 4.88 µg/ ml) and excellent bioavailability (F = 93.2%) in dog. The plasma elimination half-life of 6 hours in dog was slightly faster than obtained for ABT-770 (8.4 hours). Peak plasma concentrations of A-291518 averaged 1.25 µg/ ml, with a bioavailability of 67.6% in monkey. The plasma elimination half-life for A-291518 averaged 11.7 hours in monkey (Figure 12, left), a value approximately half that obtained for ABT-770 (23 hours).

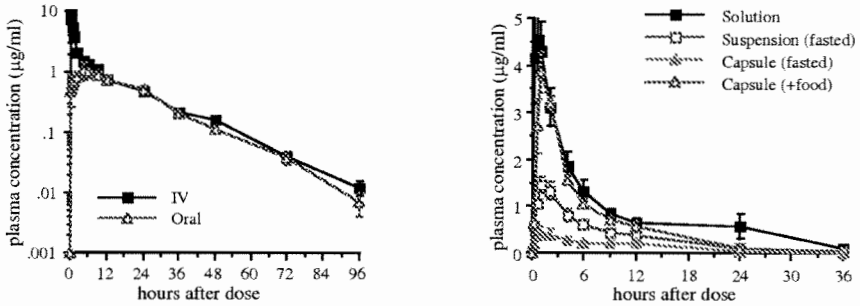


Figure 12. *Left* Plasma concentrations of A-291518 after a 3 mg/ kg IV or oral dose in monkey (Mean ±SEM, n=5). *Right* Effect of formulation and food on the plasma concentrations of A-291518 after a 3 mg/ kg oral dose in dog.

The effect of formulation and food on bioavailability following a 3 mg/ kg oral dose in dog followed trends predictable for sparingly soluble compounds (Table 13 and Figure 12, right). The highest peak plasma concentrations and bioavailability were obtained with the solution formulation of A-291518 (C_{max} = 4.88 µg/ ml, 93.2% bioavailability). Plasma concentrations of A-291518 decreased substantially following oral dosing of a suspension of the crystalline solid in 0.2% HPMC with C_{max} = 1.45 µg/ ml and 37.1% bioavailability. A further decrease in availability followed oral administration of A-291518 as a non-formulated capsule, with peak concentrations of 0.46 µg/ ml and a bioavailability of 14.9%. Administration of the non-formulated capsule with food assisted in the solubilization of A-291518, with peak concentrations of 4.10 µg/ ml and a bioavailability of 62.8%. The formulation and food effects noted with A-291518 were greater than obtained for ABT-770.

Table 13. Effect of Formulation and Food on the Pharmacokinetics of A-291518 after a 3 mg/ kg Oral Dose in Dog.

Formulation	Food?	$t_{1/2}$	C_{max}	T_{max}	AUC	F	n
Solution[a]	No	5.9	4.88	0.6	33.52	93.2	6
Suspension[b]	No	6.5	1.45	1.2	13.15	37.1	6
Capsule[c]	No	6.5	0.46	0.9	5.35	14.9	6
Capsule[c]	Yes	4.5	4.10	1.2	22.06	62.8	6

Units: $t_{1/2}$ (hr); AUC (μg•hr/ ml); C_{max} (μg/ ml); T_{max} (hr); F(%).
a. PEG 400. b. suspension (0.2% HPMC). c. non-formulated capsule; capsule formulations prepared with crystalline bulk drug.

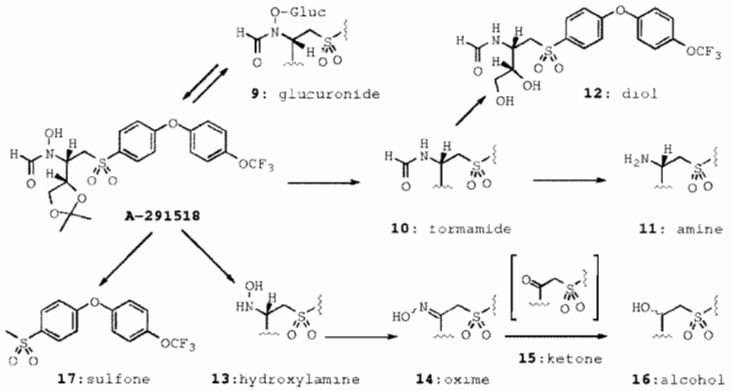
B. Metabolism

A-291518 is extensively metabolized upon multiple dose oral administration. Although the production of metabolites is minor in plasma after a single dose, at least five major metabolites have been identified following multiple dosing of A-291518 in mouse, rat and monkey. A summary of what is currently known about the metabolism of A-291518 is provided below as a prelude to a discussion of its multiple dose pharmacokinetics.

1. A-291518 Metabolism

The metabolites identified thus far for A-291518 are generally analogous to those found with ABT-770, suggesting that they arrive via a similar metabolic cascade. The metabolic pathway proposed for A-291518 in Scheme 2 is therefore based in large part on *in vitro* and *in vivo* metabolism studies with ABT-770. Most of the A-291518 metabolites identified thus far result from transformation of the retrohydroxamate moiety. This structural unit is necessary for chelation to the active site zinc atom of the MMPs, consequently none of these metabolites exhibit MMP inhibitory activity.

Scheme 2. Proposed Metabolism of A-291518.



The metabolic degradation of A-291518 is likely to be mediated, at least in part, by biliary elimination of its glucuronide 9 resulting in the potential for enterohepatic recirculation. Intestinal bacteria are thought to catalyze the conversion of A-291518 to formamide 10 under anaerobic conditions in the large intestine. This reduction may also be catalyzed by cytochrome P-450 isozymes in the liver. Loss of the formyl group of 10 gives rise to amine 11, while acetonide hydrolysis produces diol 12. Deformylation of A-291518 to hydroxylamine 13 initiates another metabolic pathway. Hydroxylamine 13 is not a major metabolite, but is suggested to give rise to alcohol 16 via through the intermediacy of oxime 14 and ketone 15. Oxidative deamination of either A-291518, formamide 10 and/ or amine 11 may also give rise to 15. Another major metabolite which could be produced by any number of pathways is sulfone 17.

As will be discussed in the following section, the importance of these metabolic pathways varies with gender and species.

C. Multiple Dose Studies

In conjunction with toxicity studies, the pharmacokinetics of A-291518 following multiple oral dosing has been evaluated in Sprague-Dawley rats and cynomolgus monkeys. Analysis of A-291518 and its metabolites was conducted in both plasma and tissues.

1. Plasma Concentrations Following Multiple Doses in Rat

Groups of rats received a 10, 30 or 100 mg/ kg/ day oral dose of A-291518 once daily for 28 consecutive days (see Section XIII for the toxicologic effects of A-291518 observed in this study). A-291518 was administered as a solution in polyethylene glycol (2 ml/ kg). Plasma samples were obtained on the first day of dosing (Day 0), the fourteenth day of dosing (Day 13) and at the end of the dosing interval (Day 27). HPLC quantification of the metabolites was sometimes confounded by overlapping peaks. This necessitated the pooling of peaks as indicated. Authentic samples of metabolites were used as identification standards for HPLC analysis, however some of the metabolites have not yet been identified. The concentration of metabolites were estimated from the A-291518 standard curve.

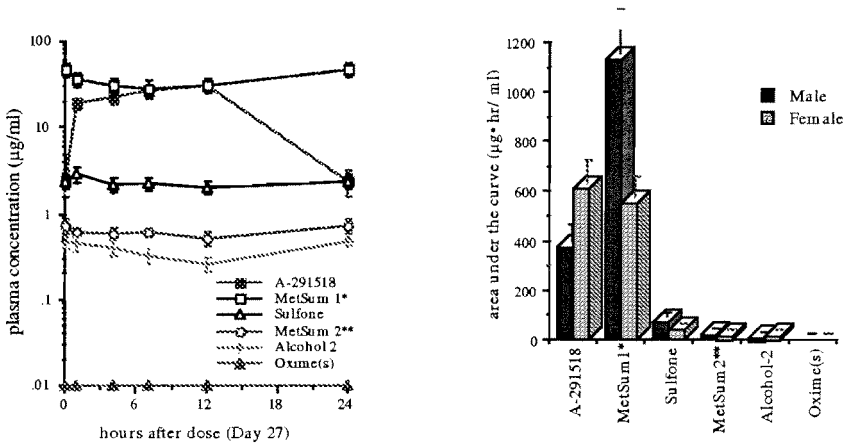


Figure 13. *Left* Plasma concentrations (mean ±SEM, n=5) of parent drug and metabolites following 100 mg/ kg daily oral dose of A-291518 for 28 days in male rats. *Right* Parent and metabolite AUC by gender (mean ±SEM, n=3).
*MetSum 1 = Amine + Unknown(s)-2 + Formamide.
**MetSum 2 = Hydroxylamine + Alcohol-1.

While parent drug was the predominant circulating component after the first dose, by the end of the four-week dosing period, multiple metabolites were found in plasma. At least eight peaks were noted in the plasma samples of rats receiving multiple oral doses of A-291518. Figure 13 (left) provides the mean plasma concentration of A-291518 and metabolites following the final dose of rats given 100 mg/ kg/ day. A-291518 reached peak plasma concentrations in excess of 30 µg/ ml approximately 6 hours after dosing. High concentrations of metabolites were also produced. The sum plasma concentration of the amine, formamide and an unknown metabolite was similar to that of A-291518 at 12 hours, but was substantially higher than parent drug at 24 hours. The sulfone metabolite plateaued at a concentration around 3 µg/ ml while a number of other metabolites did not reach concentrations above 1 µg/ ml.

The metabolism of A-291518 in rat is gender dependent. Area under the curve (AUC) values for A-291518 and its metabolites are separated by gender in Figure 13 (right). Higher concentrations of metabolites and lower concentrations of parent drug were produced in male rats. Gender specific differences in metabolite concentrations were observed in all dose groups as well as in tissue samples (Section XII.C.3). Such gender differences in the rate and/ or route of metabolic disposition of drugs in rodents are not uncommon and have a mechanistic basis in the gender specific expression of certain cytochrome P450 isozymes.[79] These differences may have toxicological implications resulting from differing exposures to parent drug and metabolites, however they can not be extrapolated to humans. In addition, clinically significant gender differences in humans is relatively uncommon.[80, 81]

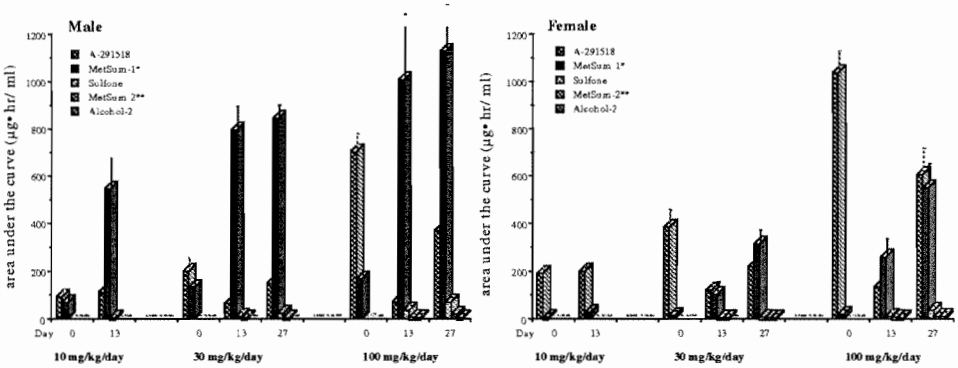


Figure 14. Effect of dose and time on AUC_{24} values (mean ±SEM, n=3) for A-291518 and metabolites following oral dosing in male rats (*left*) and female rats (*right*). The 10 mg/ kg/ day dose of A-291518 was administered for 14 days only.
*MetSum 1 = Amine + Unknown(s)-2 + Formamide.
**MetSum 2 = Hydroxylamine + Alcohol-1.

The time dependency for AUC values following 10, 30 and 100 mg/ kg/ day doses of A-291518 in male and female rats is provided in Figure 14. A comparison of the parent to metabolite AUC values produced by males (left) and females (right) clearly illustrates the gender-dependent metabolism of A-291518 in rats, metabolism being more prevalent in males. As expected, metabolite AUC values increased substantially between Day 0 and Day 13 for male rats for the 10 mg/ kg/ day dose group, while parent drug AUC values remained constant over this period. The same time interval produced a reduction in parent exposure in the 30 and 100 mg/ kg/ day dose groups. One explanation for a fall in drug exposure with time is the induction of drug metabolizing enzymes.[82] This hypothesis is supported by preliminary experiments indicating an increase in certain cytochrome P450 isoenymes (CYP2B and CYP3A in male rats, CYP3A in female rats) in liver tissues from A-291518-treated rats as detected by Western blots. The trend for a reduction in parent drug exposure for the 30 and 100 mg/ kg/ day doses does not hold between Day 13 and Day 27. In fact, a partial rebound in A-291518 exposure occurs for both dose groups. The relevance of these changes, particularly in light of the constant parent exposure produced by the 10 mg/ kg/ day dose between Day 0 and Day 13, is ambiguous. Yet, given the likelihood that A-291518 will be administrated in combination with other chemotherapeutic agents, its potential to alter human liver enzymes will require further investigation.

2. Plasma Concentrations Following Multiple Doses in Monkey

A-291518 was administered to one male and one female cynomolgus monkey at a dose of 100 mg/ kg once daily for thirteen consecutive days. The compound was prepared as a solution in polyethylene glycol (2 ml/ kg). Figure 15 (left) gives plasma concentrations of A-291518 and

metabolites over a 48-hour period following the final dose of the study. Peak plasma concentrations of A-291518 reached 13 µg/ ml. With the exceptions of two unidentified polar metabolites, most of the metabolites produced after multiple doses of A-291518 in monkeys are the same as those seen in rats. Yet the ratio of metabolites is clearly species dependent. While the formamide and unknown-2 are the major metabolites produced in rats, two polar metabolites predominate in monkeys. As was observed in rats, parent drug and metabolites reach a steady state concentration over the first 24 hours post final dose. Significantly, plasma concentrations of all analytes decline between 24 and 48 hours. Figure 15 (right) provides a comparison of AUC values for A-291518 and metabolites on Day 0 and Day 12. AUC values for several metabolites exceeded parent drug by Day 12. A 4-fold reduction in A-291518 exposure occurred over the length of the study, reminiscent of the first 14-days of the rat study. More extensive studies will be required to determine whether a reduction in A-291518 exposure occurs at lower doses.

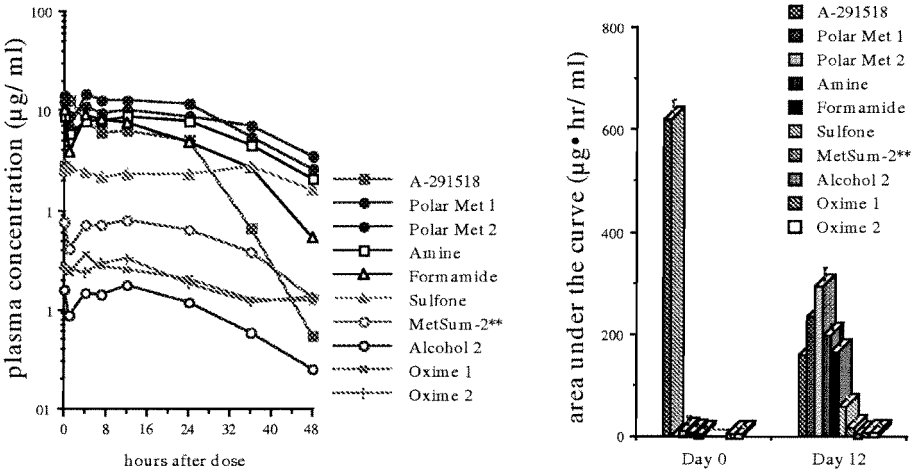


Figure 15. *Left* Mean plasma concentrations of parent drug and metabolites after 100 mg/ kg/ day oral dosing with A-291518 in monkey (Day 12) *Right* Mean (±SD, n=2) parent drug and metabolite AUC values following 100 mg/ kg/ day oral dosing with A-291518 in monkey (Day 0 vs. Day 12)
**MetSum2 = Hydroxylamine + alcohol-1

3. Tissue Concentrations Following Multiple Doses in Rat

One of the key issues regarding the development of ABT-770 was the accumulation of substantial amounts of metabolites in tissues of chronically treated animals. Millimolar quantities of ABT-770's amine metabolite were detected in lungs of rats given 100 mg/ kg/ day twice daily over 14 days. The extensive phospholipidosis observed with ABT-770 in rats and monkeys after multiple dosing was coincident with the accumulation of the amine metabolite (Section XIII.F). An assessment of the tissue concentrations of A-291518 and its metabolites was therefore included as part of the multiple dose rat study protocol. Lung, liver and kidney tissues were collected 24 hours after doses given on Day 13, Day 27 and one week following the end of the dosing period. Homogenates were extracted and analyzed by HPLC versus external standards. Assay variability was shown to be minimal based on the consistency of a number of paired samples.

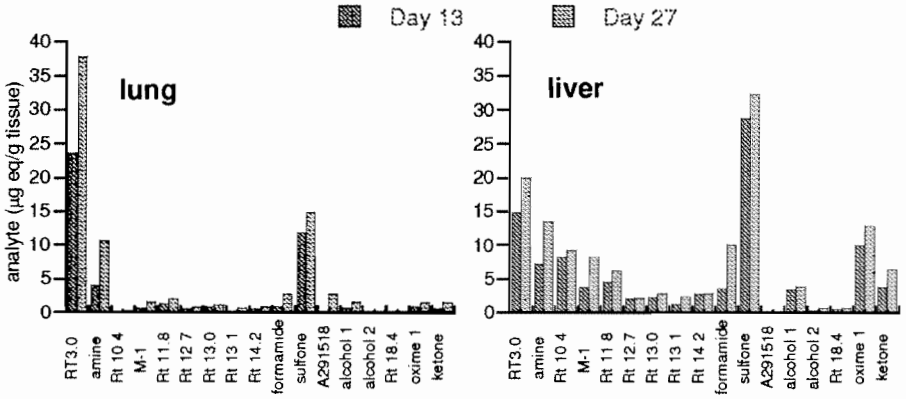


Figure 16. Tissue concentrations of A-291518 and metabolites in lung (*left*) and liver (*right*) following multiple oral doses of 100 mg/ kg/ day in rats. Rt, retention time (minutes).

The sum of A-291518-derived metabolites was less in kidney tissue than in lung and liver. Figure 16 provides the concentration of individual metabolites found in lung (left) and liver (right) tissues from male rats following 14 and 28 days of dosing. Consistent with the plasma samples, gender differences were observed, males being somewhat more prone to metabolite formation than females (data not shown for reasons of clarity). The metabolites produced in tissues are generally the same as those observed in plasma; note that the sulfone metabolite and A-291518 are poorly resolved when either analyte exceeds 1-2 µg/ g tissue. Based on these data (n=2 per time point) there appears to be only moderate increases (generally less than 2-fold) or no increase in the total metabolite concentrations between 14 and 28 days of dosing of A-291518. Even more significantly, the absolute concentration of metabolites for A-291518 were substantially less than those observed with ABT-770. None of the A-291518 metabolites were detected in concentrations higher than 40 µg/ g tissue, nearly two orders of magnitude less than the values for the amine metabolite of ABT-770. Furthermore, the concentration of all drug derived analytes increased as a function of dose with A-291518, both in lung and liver. This was not true for ABT-770 which increased greater than dose proportionally between the 30 and 100 mg/ kg/ day doses in lung (5-fold; Figure 17, left) and liver (3-fold; Figure 17, right).

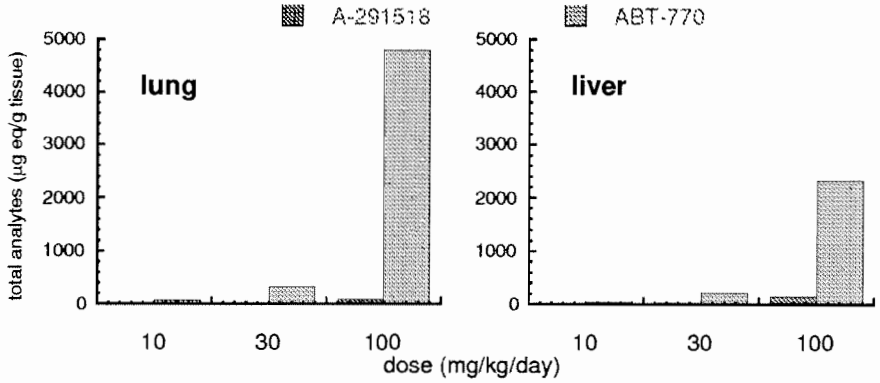


Figure 17. Concentration of total drug dependent analytes in lung (*left*) and liver (*right*) from male rats following multiple oral doses of A-291518 and ABT-770 over 28 days. The 10 mg/ kg/ day dose of A-291518 was administered for 14 days only.

From a safety perspective, the issue of metabolite accumulation is somewhat less concerning if metabolites are cleared from tissues at a reasonable rate once dosing has ceased. Figure 18 provides evidence that A-291518-derived metabolites are indeed cleared from lung and liver tissues during a one week period following 28 days of dosing. Virtually complete wash out of drug related analytes was seen over this period. Total analytes declined over the same wash out period with ABT-770, yet substantial quantities remained, particularly in lung tissue.

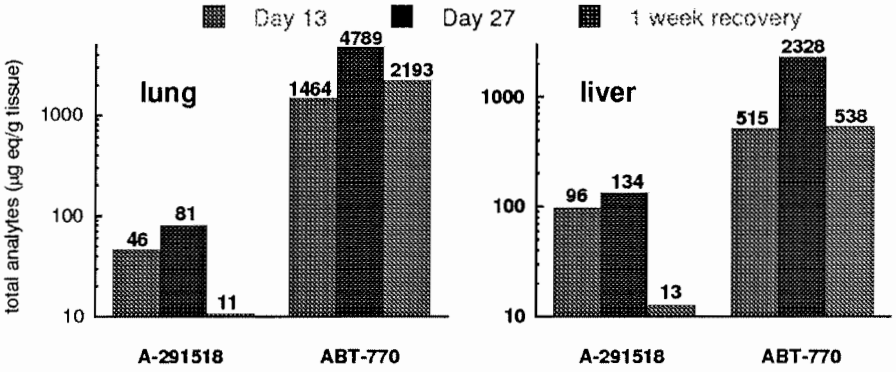


Figure 18. Concentration of total drug dependent analytes in lung (*left*) and liver (*right*) from male rats following multiple oral doses (100 mg/ kg/ day) of A-291518 and ABT-770 over 14 and 28 days and after 1 week of recovery.

4. Tissue Concentrations Following Multiple Doses in Monkey

An assessment of the tissue concentrations of A-291518 and its metabolites was included as part of the multiple dose monkey study protocol (n=2). Lung and liver tissues were collected 48 hours after a 13-day dosing period (100 mg/ kg/ day). Homogenates were extracted and analyzed by HPLC versus external standards.

The absolute concentration of metabolites for A-291518 was substantially less than those observed with ABT-770. This was true in both lung (mean 94 µg•eq/ g A-291518 versus > 812 µg•eq/ g ABT-770) and liver (mean 148 µg•eq/ g A-291518 versus > 871 µg•eq/ g ABT-770). None of the A-291518 metabolites were detected in concentrations higher than 70 µg/ g tissue, approximately 5- to 10-fold less than the values for the amine metabolite of ABT-770. The major metabolite (responsible for approximately 50% of all metabolites) was very polar and has yet to be identified. No A-291518 was detected in tissue samples 48 hours post the final dose. Due to the limited study size, no conclusion can be made as to accumulation of metabolites as a function of time or as a function of dose with A-291518. In contrast to ABT-770, the amine metabolite of A-291518 does not show any propensity to accumulate in the lung. The metabolite concentrations of A-291518 are generally higher in the liver than they are in the lung, as one might expect given that liver is the major organ of elimination for these compounds.

Overall, the comparatively small quantities of A-291518-derived metabolites in rat and monkey tissues is consistent with the benign behavior of A-291518 in toxicity studies (Section XIII.F).

XIII. Safety

A-291518 does not exhibit meaningful effects in genotoxicity, cytotoxicity and ligand binding assays. No significant CNS effects are produced by A-291518 and the compound exhibits an improved acute cardiovascular safety profile relative to ABT-770. A-291518 is well tolerated in rats treated for up to four weeks and in a small number of monkeys treated for two weeks. The maximum tolerated dose in both species was greater than the highest dose tested (100 mg/kg/day). Changes produced by A-291518 included an increase in liver weight in rats and a slight decrease in food intake in one monkey. Based on AUC values from efficacy and safety studies, A-291518 has a larger therapeutic window than ABT-770 in rodents.

A. Genotoxicity

1. Mutagenicity

A-291518 was evaluated in a bacterial reverse mutation assay for mutagenic activity (frameshift and base substitutions) as measured by the ability of the tested strains to grow under histidine-free conditions (Ames Test). The assays were conducted using *Salmonella typhimurium* strains TA-98, TA-100, TA-1535 and TA-1537 and *Escherichia coli* strain WP2uvrA- with and without an Aroclor-induced rat liver S9 metabolic activation system. The vehicle (DMSO, 50 µl/ plate) and the positive controls for each strain, non-activated and activated by S9, produced the expected findings. A dose-related three-fold increase over vehicle controls for *S. typhimurium* strains TA-1535 and TA-1537, and a two-fold increase over vehicle controls for *S. typhimurium* strains TA-98 and TA-100 and the *E. coli* strain indicate a mutagenic effect.

A-291518 was tested over a range of 1-5000 µg/ plate. Increased colony formation suggestive of a positive mutagenic event was observed, but this was later determined to be a likely artifact of the cytotoxicity present at the high test concentrations. A-291518 was therefore determined to be non-mutagenic.[83, 84]

2. Clastogenicity

A-291518 was evaluated for its ability to induce chromosome damage using the Chinese hamster V79 lung cell model in the presence and absence of an Aroclor-induced rat liver S9 metabolic activation system. Micronuclei appear when whole chromosomes lag (due to spindle disruption) or chromosome fragments appear and are not incorporated into the daughter cell nucleus. This assay has the potential to detect aneuplody inducers as well as clastogens.

Vehicle (1% DMSO) and the positive control, mitomycin C (0.5 µg/ ml), produced the expected cytotoxicity and clastogenicity. A three-fold increase of micronucleus frequency over solvent control and a concentration-dependent response are sufficient to conclude a positive response. Data are summarized in Table 14. Typically, a compound is tested to that concentration which reduces cell proliferation by approximately 50%, compared to vehicle control, or to its limit of solubility. A-291518 was tested over the concentration range of 150-190 µg/ ml; antiproliferative effects were observed at all concentrations tested. A-291518, over the concentration range tested, was not clastogenic.[85, 86]

Table 14. Clastogenicity of A-291618 in Chinese Hamster V79 Lung Cell Model.

	Clastogenicity Analysis		
	Concentration	Proliferation	Micronucleation
Compound	(µg/ ml)	% of Control	% Cells
A-291518	150	53	1.0
(lot# 543095)	160	43	0.7
	170	56	3.1
	180	42	3.5
	190	6	Too few cells
DMSO (vehicle)	1%	100	1.6 ± 0.8

B. Cytotoxicity

A-291518 was assessed for cytotoxicity on a murine tumor cell line and several human cancer cell lines (18 hr exposure) using the Alamar Blue assay (a modification of the MTT reduction assay). In non-proliferating primary cultures of rat and human hepatocytes, viability was assessed using the MTT assay after 24 hr exposure. At concentrations as high as 45 µM (>1,000-fold above the gelatinase A IC_{50} value), A-291518 had no significant effect on cell viability. As shown in Table 15, A-291518 was cytotoxic to B16 melanoma cells above 45 µM. No cytotoxicity was observed with A-291518 in human cancer cell lines at 100 µM, the maximum concentration tested. A-291518 was evaluated at higher concentrations in the MDA-435 breast carcinoma line and produced an EC_{50} value of 104 µM. In a single experiment in conjunction with a cellular assay of lamellar body formation, A-291518 was found to be cytotoxic to primary cultures of human hepatocytes only at 90 µM and was not cytotoxic (EC_{50} > 200 µM) to rat hepatocytes in primary culture. A similar separation between intrinsic potency and cytotoxicity was also observed with the other MMP inhibitors evaluated.

Table 15. Cytotoxic Effect of A-291518, ABT-770 and prinomastat on Cancer Cell Lines.

Cell Line	Cytotoxicity EC_{50} (µM)		
	A-291518	ABT-770	prinomastat
B16 (murine melanoma)	45	50	>300
MDA-435 (human breast carcinoma)	104	>300	>300
HT-1080 (human fibrosarcoma)	>100	175	>300
MiaPaCa (human pancreatic carcinoma)	>100	300	>300
Human hepatocytes	90	35	not tested
Rat hepatocytes	>200	90	not tested

C. Ligand Binding

The receptor binding affinities of A-291518 were determined at 10 µM in a battery of 76 radioligand binding assays conducted by CEREP, Inc.[87] For A-291518, the largest receptor effects observed were 39% and 30% inhibition of radioligand binding to the sigma and chloride ionophore receptors, respectively. This was considerably higher than the IC_{50} values for the reference compounds binding to these receptors: haloperidol (IC_{50} = 75 nM) and picrotoxinin (IC_{50} = 204 nM), respectively.

D. Central Nervous System Safety

A-291518 was evaluated for CNS effects in a series of standard CNS behavioral assays conducted by Phoenix International Pharmacology.[88] A-291518 had no observable effect up to 100 mg/ kg, po, in a preliminary observation test (Irwin test) and produced sedation and some reduction in muscle tone at 300 and 1000 mg/ kg. A-291518 was tested subsequently at 10-300 mg/ kg, po, in the standard behavioral tests. In these tests, A-291518 did not significantly affect any of the parameters and is considered devoid of obvious CNS activity up to 300 mg/ kg.

E. Cardiovascular Safety

The cardiovascular safety of A-291518 was assessed using an anesthetized dog model. Beagles (8.3 - 12.1 kg, n=6) were anesthetized with sodium pentobarbital (35 mg/ kg) and maintained (6 mg/ kg/ hr) at a surgical plane of anesthesia. Animals were intubated and mechanically ventilated. Electrocardiogram electrodes were placed in a lead II configuration. A Swan Ganz catheter was advanced into the pulmonary artery via the right jugular vein. A dual sensor micromanometer was placed into the left ventricle of the heart via the right carotid artery.

Each dose of drug was administered as a 30 minute intravenous infusion. Three doses were chosen to achieve therapeutic and supratherapeutic plasma concentrations as defined by the estimated minimum efficacious plasma concentrations of less than 0.5 µg/ ml (Section XI.A.3) The highest dose was projected to achieve plasma concentrations at least 30-fold higher than those deemed effective in preclinical models. Animals were monitored for 1 hour following

administration of the highest dose. Blood samples were taken at time 0 and at 15-minute intervals for the duration of the protocol.

Infusion of A-291518 at 1.0, 3.0 or 10.0 mg/ kg resulted in plasma concentrations of 2.50 ± 0.50, 5.98 ± 0.47 and 21.17 ± 1.37 μg/ ml, at the end of each 30 minute infusion interval, respectively. For plasma concentrations up to 21 μg/ ml, A-291518 produced no significant changes in mean arterial pressure, heart rate, cardiac contractility or cardiac output in anesthetized beagles. Also, A-291518 had minimal or no effect on systemic vascular resistance, pulmonary arterial pressure, electrocardiographic function, or hematocrit. The only significant effect was a small increase in pulmonary vascular resistance (compared to changes in the vehicle control) that reached 18 ± 5% above baseline midway through, and 23 ± 6% above baseline at the end of the high-dose infusion. A-291518 reached plasma concentrations of 15.22 ± 0.80 and 21.17 ± 1.37 μg/ ml during these infusion periods, respectively. Pulmonary vascular resistance tended to increase during the 60-minute post infusion period, but these changes were not significantly different from those of vehicle controls. These changes were of limited magnitude and were not sustained after treatment was terminated.

F. Toxicology

Toxicology studies conducted with ABT-770 included 2-week and 4-weeks oral GLP studies in rats[89, 90] and monkeys.[91, 92] The primary safety concerns resulting from these studies included: generalized debilitation, widespread phospholipidosis, degenerative stomach lesions in monkeys, and deaths at 3-4 times the projected clinical exposure. A high tissue burden of ABT-770 and its metabolites was also observed. Furthermore, predictive markers for toxicity were not identified. These results suggested that ABT-770 does not possess an adequate safety margin, consequently its development was curtailed in November, 1999 in lieu of a safer backup inhibitor.

1. Four-Week (with Two-Week Interim) Oral Toxicity Study in Rats

A-291518 was orally administered once daily to female and male Crl:CD(SD)BR rats at 10, 30 and 100 mg base/ kg/ day in a PEG-400 vehicle (2 ml/ kg).[93] All animals were treated for 14 or 28 consecutive days. Clinical signs, body weight, food consumption, clinical and anatomic pathology were evaluated and pharmacokinetic parameters calculated. Pharmacokinetic information including tissue concentrations of A-291518 and metabolites are reported in Section XII.C. The findings for A-291518 are summarized as follows:

- A-291518 produced no drug-related mortality or toxicologically significant clinical signs.
- No effects on body weight gain were observed. Food consumption was not significantly affected by treatment.
- No toxicologically meaningful changes in hematology or clinical chemistry indices were detected.
- A-291518 caused no meaningful macroscopic findings.
- Liver and kidney weights increased for males and females given 100 mg/ kg/ day.
- No phospholipidosis was observed. Expansion of the chondrocyte zone of hypertrophy (growth plate) was present in a dose-dependent fashion. This change was less pronounced at 4 weeks than at 2 weeks.
- Gender-related differences in the pharmacokinetic parameters were observed at all dosages administered. Females exhibited higher AUC and C_{max} values for parent drug than males on all days sampled.

A comparison of the effects produced by A-291518 and ABT-770 in rats is provided in Table 16. Significantly, the exposure of the two agents (as measured by 24-hour AUC values) are comparable after 4 weeks of dosing. The selected parameters are those that were observed to have toxicologically meaningful changes for either compound.

Table 16. Oral Toxicity Studies of ABT-770 and A-291518 in Rats.

Compound AUC_{24} (µg•hr/ ml) male/ female	Dose mg/ kg / day	Mortality total deaths	Weight Gain (g) m/ f	Liver Wt. % total body wt.	WBC (E3/ µl)	Retics (E3/ µl)	Histopathology (# affected/ # examined)
				ABT-770			
2-week	0	0	110/ 50	3.3	8.6	224	None
35/ 23	10	0	112/ 49	3.3	8.1	235	None
170/ 118	50	0	98/ 44	3.4	9.6	230	Lung - phospholipidosis (9/ 20)
463/ 452	200	8	-16/ -14	4.0	3.7	97	Lung - phospholipidosis (21/ 21) Marrow hypocellular (18/ 21)
4-week	0	0	168/ 69	3.1	8.6	175	None
40/ 41	10	0	164/ 69	3.1	8.0	130	Not Examined
113/ 116	30	0	157/ 67	3.1	9.8	185	Not Examined
439/ 409	100	3	91/ 11	3.9	3.6	122	Lung - phospholipidosis (15/ 30) Stomach necrosis (4/ 22)
				A-291518			
2-Week	0	0	38/ 25	2.6	8.0	214	None
111/ 200	10	0	44/ 24	3.2	10.0	235	Growth plate (2/ 10)
63/ 122	30	0	40/ 30	3.6	10.3	227	Growth plate (5/ 10)
73/ 138	100	0	35/ 24	4.2	10.8	305	Growth plate (9/ 10)
4-Week	0		135/ 58	3.2	8.6	170	None
-	10	-	-	-	-	-	-
148/ 222	30	0	143/ 62	3.2	5.9	196	Growth plate (1/ 10)
369/ 608	100	0	133/ 56	4.4	9.7	304	Growth plate (4/ 10)

The absence of phospholipidosis observed with A-291518 is consistent with the lower tissue concentration of parent drug and metabolites compared to ABT-770 (Section XII.C.3). The amine metabolite of ABT-770, which accumulates in excessive amounts in various tissues, causes lamellar body formation (phospholipid accumulation) in rat and human hepatocytes.[94] This attribute has been shown to be a useful predictor of compounds to cause phospholipidosis in tissues.[95, 96] A-291518 and its metabolites are less prone to accumulate in tissues and produced only marginal effects on lamellar body formation in rat and human hepatocytes.

It is important to distinguish between the joint/ bone changes observed with A-291518 relative to those produced by ABT-770 and marimastat. Synovial proliferation occurred in the joints of rats given ABT-770 while rats treated with A-291518 experienced an expansion of the zone of hypertrophying chondrocytes (growth plate). Thickening of the growth plate is considered to be a manifestation of pharmacologic activity rather than toxicity. Gelatinase B-deficient mice exhibit a similar lesion, that is, an abnormal pattern of skeletal growth plate vascularization and ossification.[97] The pathogenesis is considered to be secondary to the lack of invading capillaries into the terminal cartilage region, a prerequisite for mineralization and conversion of this tissue to osteoid. It is theorized that without capillary invasion and conversion to osteoid, the zone of hypertrophying chondrocytes continues to accumulate, thus resulting in an apparent thickening of this zone. Approximately 3 weeks after birth, gelatinase B-deficient mice develop histologically normal growth plates, suggesting compensatory mechanisms for the role of gelatinase B in the conversion of cartilage to osteoid. This is consistent with the reduction in incidence in growth plate lesions seen with A-291518 among rats treated for 4-weeks versus 2-weeks.

Joint changes have previously been characterized for rats treated with marimastat over a two week period via osmotic mini-pumps.[98] As with A-291518, marimastat produced growth plate thickening, but it also caused a number of changes not observed in A-291518-treated animals. These included subphyseal fractures, fibroplasia of the musculotendinous insertion

sites, and clinically evident impairment of motion. The steady state plasma concentrations of marimastat associated with these changes was determined to be 490 nM, less than the 1.5 μM (males) and 7 μM (females) trough concentration produced by oral administration of A-291518 over 28 days.

The changes in liver weight produced by A-291518 may be a consequence of the potential for microsomal enzyme induction (Section XII.C.1). A general association between hepatomegaly and the induction of drug-metabolizing enzymes has been established in rodents.[99] Less well established, however, is the correlation between liver weight increases and evidence of chemically-induced liver injury.[100] In the absense of changes in liver morphology and no meaningful elevations in serum liver enzymes, evidence of liver injury induced by A-291518 is lacking.

2. Two-Week Oral Toxicity Study in Cynomolgus Monkeys

A-291518 was administered by oral intubation once daily to young adult monkeys (1/ sex) at 100 mg/ kg/ day in a PEG-400 vehicle (2 ml/ kg).[101] The vehicle control group included two females and no males. All animals were treated 14 consecutive days. Each animal received 6-15 ml of PEG-400 daily based on body weight; this volume of PEG-400 was expected to produced diarrhea. Clinical signs, body weight, food consumption, clinical and anatomic pathology were evaluated and pharmacokinetic parameters calculated and tissue drug levels determined. Pharmacokinetic information including tissue concentrations of A-291518 and metabolites are reported in Section XII.C.2. The findings for A-291518 are summarized as follows:

- A-291518 produced no drug-related mortality or toxicologically significant clinical signs.
- No effect on body weight gain was noted. Food intake was slightly decreased in the female monkey relative to control.
- No toxicologically meaningful changes were found in hematology or clinical chemistry indices.
- A-291518 caused no meaningful macroscopic findings.
- No toxicologically meaningful histopathologic changes were observed, including no evidence of phospholipidosis.

Bearing in mind the limited number of animals in this study, treatment with A-291518 was well tolerated over the two-week treatment period, with little evidence of the target organ toxicities observed with a high dose of ABT-770 (debilitation, pulmonary phospholipidosis, and degenerative changes in the stomach - Table 17). Furthermore, liver changes observed in a previous pilot study with A-291518 suggestive of lipid accumulation were not confirmed by this study. Definitive conclusions about the relative safety of A-291518 versus ABT-770 in monkeys are confounded by the fall in plasma concentrations seen with A-291518 over the two-week dosing period.

Table 17. Oral Toxicity Studies of ABT-770 and A-291518 in Monkeys.

Compound AUC_{24} (μg•hr/ ml) Day 0/ Day 13	Dose mg/ kg / day	Mortality incidence	Weight Gain (kg) m/ f	Bile Acids μmol/l	Stomach Necrosis incidence	Other Histopathology (# affected/ # examined)
			ABT-770			
	0	0	+0.29/ -0.01	4.6	0/ 10	None
115/ 344	30	0	-0.00/ +0.06	7.1	0/ 6	None
220/ 550	100	0	-0.08/ -0.14	12.8	4/ 6	None
433/ 1,060	300	4	-0.33/ -0.26	28.1	9/ 10	Lung phospholipidosis (3/ 6)
			A-291518			
	0	0	-- / -0.07	2.9	0/ 2	None
620/ 159	100	0	+0.16/ -0.10	10.7	0/ 2	None

G. Therapeutic Window

The potential therapeutic window afforded by A-291518 can be estimated by comparing the drug exposure necessary for efficacy in experimental models to drug exposure obtained in safety studies. A compilation of efficacy and safety exposures (expressed as AUC values) and the ratio of the two are shown in Table 18.

Table 18. Comparison of Drug Exposure from Efficacy and Safety Studies.

Inhibitor model	Efficacy			Safety							
				4-week rat				2-week monkey			
	dose[a] bid	PI[b]	AUC[c]	dose[a] qd	AUC[d]	therapeutic ratio[e] B16	therapeutic ratio[e] HT1080	dose[a] qd	AUC[d]	therapeutic ratio[e] B16	therapeutic ratio[e] HT1080
ABT-770											
B16	6	38	30	10	40	**1**	**0.6**	30	344	**11**	**5**
HT1080	60	25	70	30	115	**4**	**1.6**	100[f]	550[f]	18[f]	8[f]
				100[f]	429[f]	14[f]	6.1[f]	300[f]	1060[f]	35[f]	15[f]
A-291518											
B16	6	33	2.6	30	185	**71**	**8**	100	159	**61**	**7**
HT1080	20	38	22	100	488	**188**	**22**				

a. mg/ kg/ day
b. Percent inhibition of control at 2 g.
c. Area under curve (μg•h/ ml) over 24 h, estimated by doubling 12 h values from bid studies.
d. Mean of male and female area under curve (μg•h/ ml) over 24 h from safety studies.
e. AUC_{safety}/ $AUC_{efficacy}$.
f. associated with drug-related toxicity.

From the tabulated values it is apparent that the exposure required to achieve efficacy, and consequently the therapeutic index, is model-dependent. Thus both ABT-770 and A-291518 were less effective (required higher AUCs) in the HT1080 model than in the B16 model. This difference could result from differential roles of MMPs in the two models. Alternatively it may reflect differences in the two mouse strains (SCID and C57) used in these studies. The issue is further complicated by differences in exposure across species in the two safety studies (e.g., A-291518 had a higher exposure in rat than monkey). Because of these considerations it is difficult to define a precise therapeutic window. Estimates of the experimental therapeutic index for A-291518 cover a range from 7 (efficacy with HT1080 and safety based on the primate data) to 188 (B16 efficacy and rat safety). It is important to recognize that these ratios represent lower limits since A-291518 cleared all doses examined so far. These estimates will become more precise as exposure in additional models is assessed and when the maximum tolerated dose is established in different species. However, in spite of these uncertainties it is clear from the above results, especially considering the significant rodent toxicity observed with ABT-770, that A-291518 potentially offers a substantial improvement in therapeutic index.

XIV. Alternative Indications

There are a wide variety of pathological conditions in which excessive MMP activity has been implicated. Indications in which selective inhibitors may prove to be efficacious include Crohn's disease (IBD), macular degeneration, periodontal disease, congestive heart failure, neuroinflammatory diseases, and glomerulonephritis. MMPs are thought to contribute to atherosclerotic plaque weakening and rupture. Interest in the development of MMP inhibitors for the treatment of osteo- and rheumatoid arthritis is particularly high. This is based largely on the overexpression and co-localization of MMP-13 (collagenase-3) in cartilage of arthritic joints.[11] Similarly, inducible expression of MMP-13 in joints of mice results in cartilage breakdown and joint destruction similar to that observed in human arthritic joints.[102] Observations from the published literature that support these and other indications are summarized in Table 19.

Table 19. Potential Disease Targets for MMP Inhibitors.

Indication	Relevance of MMPs
Osteoarthritis	MMP inhibitors prevent the break down of cartilage explants and demonstrate beneficial effects in animal models of osteoarthritis. Several are now in clinical development for the treatment of osteoarthritis.[103] [104, 105]
Rheumatoid Arthritis	MMP inhibitors decreased bone degradation in a rat model of arthritis[106] and decreased the severity of collagen-induced arthritis in mice (Project Team results).
Macular Degeneration (MD)/ Proliferative diabetic retinopathy (PDR)	Angiogenesis is a key component of both MD and PDR. The antiangiogenic activity of MMP inhibitors, the levels of gelatinases in the vitreous [12], the occurrence of TIMP mutations [107, 108], and MMPs in the choroidal neovascular membrane [109] suggest a therapeutic potential in macular degeneration. Prinomastat is currently undergoing a Phase II clinical study for the treatment of MD.[110, 111] Batimastat is being evaluated for the treatment of recurrent pterygia. [112]
Periodontal Disease	MMP activity has been implicated in the irreversible destruction associated with periodontitis and peri-implantitis. [113] Chemically-modified tetracyclines (CMT) have been shown to decrease MMP-8 expression and bone loss in animal models of periodontitis [114] and periostat (low dose doxycycline) was approved for the treatement of periodontal disease in 1998. [115]
Atherosclerosis/ Aneurysms	MMP-9 activity correlates with the rupture of atherosclerotic plaques, suggesting that inhibitors may prevent plaque ruptures and aneurysms.[116-118] The involvement of MMP-2, MMP-9, and MMP-13 have been implicated in the progression of abdominal aortic aneurysms.[119-121]
Congestive Heart Failure (CHF)	The development of CHF is associated with a down-regulation of TIMPs and an up-regulation of MMP-9.[122] In a swine model of CHF, selective MMP inhibitors limited disease progression. [123]
Neuroinflammatory Diseases	MMPs (particularly MMP-9) have been shown to be increased in the cerebral spinal fluid (CSF) in models of multiple sclerosis (MS) and peripheral neuritis. MMP-2 and other MMPs are associated with macrophage infiltrates in disease plaques. MMP inhibitors demonstrate beneficial effects in animal models of MS concomitant with a decrease in the gelatinase activity in the CSF. [124]
Crohn's Disease (IBD)	The gelatinases from infiltrating neutrophils have been associated with the progression and severity of Crohn's disease.[105]

Indication	Relevance of MMPs
Glomerulonephritis	MMP-2 peaks in experimental glomerulonephritis and MMP inhibitors attenuates anti-Thy1.1 induced nephritis.[125]

XV. Competition

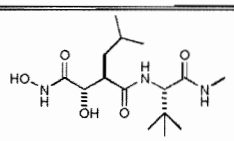
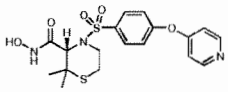
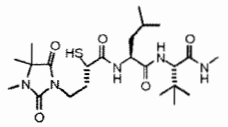
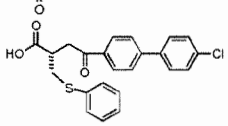
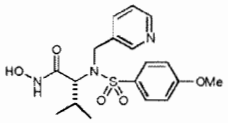
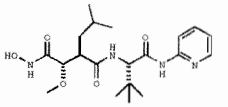
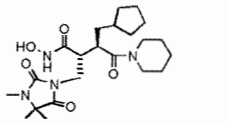
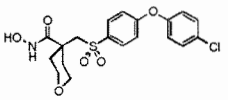
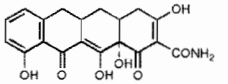
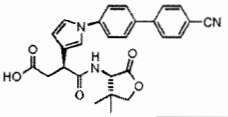
While the competition in the MMP inhibitors field is intense, no compound has yet been approved. Clinical trials for MMP inhibitors have been hampered by dose limiting joint toxicity. Enhanced survival was not observed in pancreatic cancer patients given marimastat alone or in combination with gemcitabine. In contrast, marimastat produced a trend for greater survival among gastric cancer patients, which was statistically significant in patients without evidence of metastasis. These results suggest that MMP inhibitors may be more appropriate for use in earlier stage disease. The selectivity of A-291518 suggests that it may avoid the dose-limiting joint toxicity observed marimastat and prinomastat, thus expanding the range of tolerated doses and enhancing the likelihood of demonstrating clinical efficacy.

A. MMP Inhibitors in Clinical Development

The MMP inhibitors field continues to be extremely competitive. More than 30 firms have filed patent applications covering small molecule MMP inhibitors over the past 5 years. Companies with compounds in clinical development for the treatment of cancer include British Biotechnology/ Schering, Agouron/ Warner Lambert and Bristol-Myers Squibb/ Chirosciences. Other companies have focused on the development of MMP inhibitors for alternative indications, primarily connective tissue diseases such as arthritis. A list of orally active MMP inhibitors which have been assessed in clinical studies is provided in Table 20.

Exhibit A
Part 4

Table 20. MMP Inhibitors in Clinical Development.

MMP Inhibitor Company	Structure	Development Status; Indication	Metalloproteinase Selectivity IC_{50} (nM)[a] MMP-1	MMP-2	TACE	Clinical Joint Toxicity
marimastat British Biotech/ Schering		Phase III; cancer	broad spectrum 0.85	0.50	1.8	yes
prinomastat Agouron/ Warner Lambert		Phase II/ III; cancer	moderately gelatinase selective 5.7	0.048	7.9	yes
BMS-275291 Bristol-Myers Squibb/ Chirosciences		Phase II; cancer	broad MMP; no TACE (9)	(41)	(--)[b]	yes
tanomastat (formerly BAY12-9566) Bayer		Withdrawn	highly gelatinase selective >10,000	120[c]	>100,000	no
CGS 27023A Novartis		Withdrawn	broad spectrum 43	12	120	yes
solimastat British Biotech/ Schering		Phase Ib; cancer	broad spectrum (10)	(80)	(140)[d]	un-known
Trocade Roche		Phase II/ III; arthritis	collagenase selective (3)	(154)	(--)[b]	un-known
RS-130830 Roche Biosciences		Phase I; arthritis	highly gelatinase selective (590)	(0.2)	(--)[b]	un-known
metastat; CollaGenex		Phase I; cancer	indirect inhibitor (30,000-50,000)		(--)[b]	un-known
AG3433 Agouron/ Warner Lambert		pre-clinical; cancer	highly gelatinase selective (13,000)	(0.9)	(--)[b]	un-known

a. IC_{50} values in parentheses are literature values

b. Compound lacks inhibition of TNF release in a cellular assay. Enzyme inhibitor assay data has not been reported.

c. The gelatinase A IC_{50} value for tanomastat determined by the Project Team is about 10-fold higher than the Ki's reported by Bayer. The primary screening assays at Bayer, described in their patent application, are carried out at pH 6.5. Carboxylic acid MMP inhibitors exhibit more potency when assayed under acidic conditions, whereas hydroxamate inhibitors have little pH dependence on their activity. In comparative assays at pH 7.4, the Abbott compounds are 30 to 150-fold more potent than BAY 12-9566 as inhibitors of gelatinase A. Although tumor tissues are generally more acidic than the surrounding stroma, pH measurements of various tumors have shown that the shift is of small magnitude (mean pH 7.0-7.2 relative to pH 7.4 in normal plasma).[126]

d. IC_{50} for inhibition of TNF release in a cellular assay. Enzyme inhibitor assay data has not been reported.

1. British Biotechnology; marimastat & solimastat

Results from a Phase I clinical trial of marimastat in healthy volunteers indicated that the drug is well absorbed with an estimated bioavailability of approximately 70%. Its half-life is between 8 and 12 hours, consistent with twice daily dosing. Plasma levels are approximately 2- to 3-fold higher in advanced cancer patients than in healthy individuals, presumably due to differences in rates of metabolism/ elimination.[59, 127, 128] Since efficacy studies have thus far been conducted in advanced cancer patients, there exists the possibility that the drug will be under-dosed in patients with less aggressive disease.[1]

The first efficacy studies with marimastat were designed based on the hypothesis that concentrations of tumor markers in the blood are related to the stage of the disease. Based on this assumption, it was thought that changes in the rate of rise of tumor marker concentrations with time (e.g., PSA for prostate cancer) could be used as a measure of marimastat's biological activity, and would provide an indication of therapeutic potential and optimal dose. In a series of trials involving various types of cancers, patients were selected who exhibited at least a 25% increase in the rate of rise of plasma cancer antigens over the month prior to initiation of the therapy.[129-132] [133] Marimastat treatment significantly reduced the rate of rise of all antigens examined in a dose dependent manner. More than 50% of the patients exhibited a diminished rise in antigens and the rate of antigen level rise rebounded seven days after the drug was discontinued. These changes were correlated with a decrease in tumor burden by CT scan in pancreatic cancer [129, 134, 135] and to an increase in survival in ovarian and pancreatic patients. [59, 132] The design and relevance of these trials has been criticized by the medical community, as it has been argued that the fall in the rate in rise of cancer antigens may merely reflect the normal marked variation in levels observed over a short interval.[2] British Biotechnology scientists have recently shown measurement of cancer antigens to be relevant in a pre-clinical tumor growth model.[136]

Doses of marimastat used in Phase III studies have been restricted to 5, 10, and 25 mg, bid due to the poly-arthralgia mentioned in Section V.C.[137] Approximately 60% of patients given a 10 mg, bid dose experience the side-effect within 1-5 months. These symptoms of joint toxicity typically do not responded well to NSAID treatment, but are reversed when patients are removed from therapy. While these "dosing holidays" have allowed treatment to continue, they have required highly customized schedules adjusted to each patient's tolerance of pain. Sensitivity to these joint effects is often quite variable; some patients have become irreversibly debilitated while others are more resistant.

Results from a randomized Phase III study comparing marimastat to gemcitabine in patients with non-resectable pancreatic cancer have been reported.[138] While the primary endpoint of survival was not achieved, 12 month survival for the 25 mg marimastat group was equivalent to that for patients treated with gemcitabine. Both of these groups fared better than patients given lower doses of marimastat. Marimastat (10 mg, bid) has also been assessed in combination with gemcitabine in a Phase III trial involving 239 advanced pancreatic cancer patients.[137] The combination of marimastat and gemcitabine failed to show a significant improvement in survival relative to gemcitabine alone. However a trend indicating that patients with less extensive disease responded better to marimastat was observed.

Encouraging results from a multi-center Phase III clinical trial involving patients with inoperable gastric cancer have recently been reported.[139] This trial compared survival among 369

patients treated with either 10 mg marimastat twice daily or placebo. The pre-defined clinical cut-off was 85% mortality in one of the treatment groups. This occurred first with patients receiving placebo at which point 22.7% of the marimastat-treated patients were alive. While the survival benefit did not achieve statistical significance at this original cut-off ($p = 0.084$), 25 of the 35 patients surviving to a second cut-off were marimastat-treated ($p = 0.048$). Furthermore, a survival benefit in favor of marimastat was seen in 101 patients without metastases at the original clinical cut-off ($p = 0.033$). These results are consistent with a Phase II study involving gastric cancer patients reported earlier.[140] Ten of 31 patients that completed the 28 day treatment period showed an increase in fibrotic cover of the tumor as assessed by endoscopy and 8 demonstrated a reduction in hemorrhagic appearance. Based on these results, British Biotechnology officials have acknowledged their intention to pursue accelerated approval for marimastat for the treatment of gastric cancer.

A collaboration between British Biotechnology and Schering-Plough on the development of marimastat has recently been announced.[141] Seven other randomized pivotal trials with marimastat are currently ongoing in small cell lung cancer, non-small cell lung cancer, breast cancer, ovarian cancer and glioblastoma. Most will assess the efficacy of marimastat in combination with standard cytotoxic agents. Phase I studies indicate that marimastat is well tolerated under these conditions.[142, 143] Results from several of these studies are expected within the next 9 months.

Another compound in early stage development is solimastat (formerly BB-3644) which British Biotechnology scientists claim is a broad spectrum MMP inhibitor with enhanced potency against TACE relative to marimastat.[141] Despite its broad spectrum profile, solimastat is also claimed to be less prone to cause joint toxicity in laboratory animals.[137] Solimastat has recently completed single dose Phase I studies in healthy volunteers and its tolerability will be evaluated in phase Ib studies in colon cancer patients. British Biotechnology has previously announced its intentions to develop solimastat for the treatment of multiple sclerosis. The structure of solimastat given in Table 20 has not yet been confirmed.

2. Agouron/ Warner-Lambert; prinomastat and AG3433

Phase I clinical trial results for prinomastat in patients with advanced lung, prostate, kidney and colorectal cancers have been reported.[62] The compound given at doses of 5 mg, bid to 100 mg, bid produced disease stabilization in more than 25% of the 47 patients who were treated for periods of 16 to 40 weeks. A Phase I trial of prinomastat dosed in combination with mitoxantrone/ prednisone in prostate cancer patients revealed no safety concerns, although arthralgia was attributed to the drug at higher doses. The side effect was managed by discontinuation of therapy for 2-4 weeks after which patients were given lower doses.

Toxicities besides arthralgia/ myalgia were minimal when prinomastat was dosed in combination with paclitaxel/ carboplatin in a Phase I study.[144] The compound is currently being assessed in several Phase III studies as part of first-line therapy in combination with cytotoxic agents. One study is designed to evaluate the safety and efficacy of prinomastat in patients with advanced non-small cell lung cancer in combination with paclitaxel/ carboplatin relative to cytotoxic therapy alone. Another study will assess the combination of prinomastat and gemcitabine/ cisplatin. Prinomastat is also being evaluated in advanced hormone-refractory prostate cancer patients in combination with mitoxantrone and prednisone. These studies will assess the effect of prinomastat at doses of 5, 10 and 15 mg, bid. Joint toxicity precluded the examination of a 25 mg, bid dose.[145]

Preclinical pharmacokinetics studies indicate that prinomastat reaches therapeutic concentrations in ocular tissues following oral administration. Given its pre-clinical antiangiogenic effects (Section XI.C) and the strong evidence linking MMPs activity with age-related macular degeneration (AMD), a Phase II clinical trial in patients with wet AMD is currently underway. This will involve a randomized, double-blind, placebo-controlled study of 100 patients with efficacy assessed by changes in visual acuity.[110, 111]

The structure of a second-generation inhibitor from Agouron designed to avoid fibroblast collagenase inhibition has recently been disclosed.[146] AG3433 is a succinate-derived carboxylic acid which is substantially more gelatinase selective than prinomastat although it is also more highly protein bound (97.8% in human plasma versus 80% for prinomastat). According to Agouron scientists, AG3433 is currently undergoing "advanced preclinical evaluation" with the intention of initiating Phase I studies in 2000.

3. Bristol-Myers Squibb/ Chirosciences; BMS-275291

MMPs other than the gelatinases have been shown to contribute to tumor progression[47, 147] If joint toxicity is mediated by metalloproteinases outside of the MMP family, broad spectrum MMP inhibitors may prove to be more efficacious than selective agents. This hypothesis is being tested clinically by Bristol-Myers Squibb through a collaboration with Chirosciences on the development of BMS-275291 (formerly Chiroscience D-2163). This compound exhibits broad MMP inhibition while having no inhibitory activity versus TACE, thus distinguishing it from compounds like marimastat. Scientists at Bristol-Myers Squibb postulated that BMS-275291 would be free from joint effects since it did not cause lesions in a marmoset model of joint toxicity. While a Phase I dose-escalation trial in healthy volunteers revealed dose-proportionality in plasma concentrations,[148] a multiple dose Phase I studies in advanced cancer patients revealed grade 1 myalgia/ arthralgia in 7 of 28 patients and grade 2 in 3 of 28 patients.[66] This data suggests that inhibition of TACE itself may not mediate joint effects, yet inhibition of related, perhaps unknown metalloproteinases can not be ruled out.

4. Bayer; tanomastat (formerly BAY 12-9566)

Until recently, Bayer was developing tanomastat (formerly BAY 12-9566) simultaneously for the treatment of cancer and osteoarthritis.[149, 150] This compound exhibits modest potency against gelatinase A (120 nM, IC_{50}) and is marginally active against gelatinase B (1,600 nM, IC_{50}). Tanomastat lacks inhibitory activity against fibroblast collagenase and TACE. Phase I studies in cancer patients indicate that the compound produces high plasma levels after oral dosing; a 800 mg, bid dose resulting in steady-state plasma concentrations of 142 µg/ ml.[151] Less than proportional increases in steady state concentrations were observed at doses above 100 mg, qd, suggesting saturable drug absorption.[152] The elimination half-life of tanomastat is exceedingly long (between 3 and 5 days) and the compound is highly protein bound (>99.9%). In Phase I studies in cancer patients with refractory solid tumors, 14 of 27 patients treated with tanomastat had no tumor progression for at least four months and five patients had disease stabilization for 7 to 11 months. Four of these patients have survived for more than one year. Despite these encouraging results, tanomastat had no effect on surrogate markers of disease progression in at least one Phase I trial, in which plasma VEGF/ bFGF and urine pyridinoline/ deoxypyridinoline concentrations were measured.[153] The compound produced no consistent effects on plasma gelatinase A or gelatinase B concentrations, although plasma concentrations of TIMP-2 tended to increase in a dose-dependent manner.[151] Dosed over extended periods, tanomastat produced no observable musculoskeletal effects.

All clinical trials with tanomastat were terminated last September when interim analysis of a Phase III trail in small-cell lung cancer revealed greater mortality and tumor progression among patients receiving the drug (800 mg, bid) relative to those on placebo.[154] Two hundred patients were assessed for the interim analysis and a 30% difference in survival rate ($p < 0.05$) was observed between the two groups.[155] The trial was intended to compare tanomastat with placebo in patients who had previously received best standard treatment. As a precautionary measure, ongoing trials involving non small-cell lung, ovarian and pancreatic cancer patients were halted. A Phase III trial comprising 1,800 osteoarthritis patients was also suspended.

An explanation for the negative effect of tanomastat in small-cell lung cancer patients is not obvious. The compound was generally well-tolerated in multiple-dose Phase I studies, with reversible increases in transaminases and thrombocytopenia being the most notable side-effects.[156] Bayer scientists have been cautious about speculating on the small-cell lung cancer

results before a thorough analysis of the data has been made, particularly to check for patient imbalances or differences in toxicities between the groups.[155]

At this point the question remains as to whether the negative findings for tanomastat are real, and if so, whether they are specific to the compound or to the small-cell lung cancer patient population. The expression of MMPs has been examined by immunohistochemical methods in tumor tissue specimens from small-cell cancer patients.[157] Positive staining was observed for most MMPs, *with the exception* of gelatinase A suggesting that this class of cancer patients may have been an inappropriate choice to test the efficacy of tanomastat. The lack of gelatinase A expression in these biopsies does not, however, address the apparent enhancement of disease progression among treated patients. It is important to reiterate that tanomastat is a marginally potent inhibitor which is highly protein-bound. The Project Team was unable to demonstrate efficacy with this agent in a least one tumor model which respond to marimastat, prinomastat and A-291518. The high steady-state plasma concentrations produced by an 800 mg dose given twice daily (142 µg/ ml) may not be appropriate for cancer patients in advance stages of the disease.

5. Novartis; CGS 27023A

CGS 27023A mimics marimastat in exhibiting broad spectrum MMP inhibition, although it is somewhat less potent. Results from Phase I studies with CGS 27023A have been reported in abstract form.[158] In 36 patients with advanced solid tumors, CGS 27023A given at doses ranging from 150 mg, bid to 600 mg, tid produced plasma concentrations of more than ten times the IC_{50} values for MMP-1, MMP-3 and MMP-9. Seven patients had stable disease and remained on treatment for more than 12 weeks. In addition to the arthralgia/ myalgia typical of broad spectrum MMP inhibitors, CGS 27023A also produced a maculopapular rash in 5 of 22 patients given doses > 300 mg, bid. While an official announcement has not been made, the compound is listed as terminated on the Novartis website.[159]

6. Roche; Trocade and RS-130830

The uncertainty surrounding the cause of MMP inhibitor-induced joint toxicity is exemplified by the development of two compounds with vastly different selectivity profiles by sister divisions of Roche. RS-130830 from Roche Biosciences is highly selective for the inhibition of the gelatinases over fibroblast collagenase.[160] This compound is also a potent inhibitor of collagenase-3, an enzyme produced by chondrocytes in cartilage. RS-130830 exhibits sub-nanomolar inhibition of collagenase-3 (0.52 nM, IC_{50}) and is efficacious in several *in vivo* models of osteoarthritis. According to Roche Biosciences scientists, inhibition of fibroblast collagenase is not necessary for efficacy in these models and should be avoided to prevent joint toxicity. RS-130830 is currently entering clinical development for the treatment of osteoarthritis.

An alternative point of view has been championed by the discoverers of Trocade at Roche Discovery Welwyn. They speculate that inhibition of sheddases such as TACE mediates the MMP inhibitor-induced joint toxicity and that fibroblast collagenase inhibition is beneficial in reducing helical collagen cleavage associated with cartilage degradation. Trocade is moderately selective for the inhibition of fibroblast collagenase relative to gelatinase A (3 nM vs. 154 nM, IC_{50}). Similar to RS-130830, this compound prevents structural damage after oral treatment in a mouse model of osteoarthritis.[161] Trocade was well tolerated following single doses of 10 to 150 mg given orally to healthy volunteers and is currently undergoing a double-blinded, Phase II/ III trial in arthritis patients.[162] Preliminary results from this trial were recently presented and reveal that some patients have experienced joint effects, although none were severe enough to require a dosing holiday.[163] At this time, it is not known whether these effects were drug related.

7. CollaGenex; metastat

Metastat is a member of the tetracycline class of MMP inhibitors. This class of inhibitors differs from the inhibitors discussed thus far in that they inhibit MMP activity by altering MMP conformation (presumably through an interaction with MMP structural calcium) rather than binding at the active site zinc atom.[164] Metastat has shown efficacy in preclinical models of

tumor metastasis.[165] However, the mechanism of action is an ongoing subject of investigation, since the compound also inhibits iNOS, COX-1 and COX-2 expression.[166] Results from a Phase I study in 15 patients with solid tumors have been reported in abstract form.[167] Three dosage levels (36, 50 and 70 mg/ m^2) were used and preliminary pharmacokinetic results suggest that metastat is well absorbed with a half-life of approximately 3 days. The most significant side effects observed were grade 1, 2 and 3 photosensitivity. Steady-state concentrations ranged from 2.5 to 11 µg/ mL. Preclinical models suggested that concentrations of 1.8 to 12 µg/ mL are required for efficacy.

XVI. Follow-up Strategy

From an intellectual property perspective, the MMP inhibitors field is quite mature. Over the past 10 to 15 years a steady stream of patent applications covering a range of structural classes have been published. Novel compositions of matter, particularly within the succinyl and biaryl hydroxamate classes, are now scarce. As described in Section VIII, the biaryl ether retrohydroxamate class of MMP inhibitors provides Abbott with a patent niche within this very crowded field. The attributes and liabilities of this class have been fully explored by the Project Team; more than 390 retrohydroxamates were synthesized and extensively characterized over a 2-year period. While exceptions are always possible, it seems unlikely that the properties of A-291518 can be vastly improved upon through further SAR within this series. Backup compounds to A-291518 could be readily identified, yet they are apt to possess very similar properties. The opportunity to pursue other lead structures, generated through NMR screening or by other means, remains as a viable option, yet these endeavors must be viewed relative to other activities within Cancer Research. At this point no further effort is being applied to the discovery of novel MMP inhibitors by the Project Team.

XVII. Risk Assessment

Notwithstanding the advantages of A-291518 relative to the competition discussed above, the key issues of clinical efficacy and safety remain as obvious uncertainties. Yet the intense competition to market the first MMP inhibitor speaks to the enthusiasm for the approach (Section XV). It should be mentioned, however, that the concept of chronic cytostatic therapy for the treatment of cancer remains to be validated and the design of definitive clinical trials remains problematic. While Abbott can take advantage of the clinical results generated by its competitors in the MMP inhibitors field, combination therapies with conventional cytotoxic drugs will need to be determined empirically. Although inhibition of tumor growth with MMP inhibitors has been demonstrated in animal models and normal cells appear to be unaffected, safety and efficacy must be proven by clinical studies in humans.

XVIII. Key Objectives and Plans for Development Team

The primary objective of the Transition Team will be to assess key attributes of A-291518 that would justify a recommendation for further development or discontinuation. Key attributes for a viable compound include: acceptable animal toxicity profile following chronic administration, favorable systemic exposure in humans after oral administration, and acceptable safety profile in cancer patients following chronic dosing (30 days or more). Therefore, the development activities during the transition period will be limited to two Phase I studies (one single dose and one multiple dose), and the toxicology and formulation activities needed in support of Phase I. It is not the objective of the transition program to demonstrate proof of efficacy of this new class of compounds, which most likely would be better assessed during Phase II and/ or Phase III studies.

The first Phase I single escalating dose study would be conducted in healthy volunteers. This is based on the assumptions that the therapeutic dose for cytostatic compounds, such as A-291518 are well below the MTD, thus it would have an acceptable safety margin. This approach would allow the Transition Team to rapidly assess (8 weeks) the pharmacokinetics of the backup MMP inhibitor. Furthermore, this study would assist in selecting, a near therapeutic level, starting

dose for the Phase I multiple dose study in cancer patients. This would help achieve the objectives of the multiple dose study with fewer dose escalations, thereby significantly reducing study duration.

A decision to proceed to the second Phase I, multiple dose (30 days) study in advanced cancer patients will be made if the results of the single dose study indicates good systemic exposure after oral administration and adequate safety. This multiple dose study will establish the pharmacokinetics in patients and will allow for an evaluation of metabolite formation/ elimination. More importantly, this study will assess the safety profile following chronic administration, which is the expected use for a cytostatic agent. Patients completing the 30-day dosing period will be given the option of continuing therapy indefinitely. This will provide a definitive assessment of joint effects and may furnish anecdotal evidence of efficacy.

Development activities throughout the transition period will be confined to those required to support the two Phase I studies: bulk drug synthesis with minimal process research by D45L, stability support of bulk drug and formulation, toxicology studies up to 1 month in two species and genotoxicity testing. After evaluation of regulatory requirements in U.S. and ex-U.S., Phase I studies will be conducted in the Netherlands where only ethics committee approval is required.

Several Go/ No-Go decision points have been incorporated into the transition program which has an overall objective of making timely recommendations for continuation of development, development of another compound, or program termination. The key Go/ No-Go points are related to (1) preclinical toxicity before Phase I, (2) pharmacokinetic profile from a single dose study, and (3) safety in patients from a multiple dose Phase I study. Should a recommendation be made to continue development, the Transition Team would propose a plan to rapidly obtain approval. As development activities during the transition period are limited, a gap of 6 months is to be expected between completion of Phase I and starting of Phase II to accommodate bulk drug synthesis, process research and formulation work to support Phase II. However, this gap could be minimized if pre-clinical activities resume before completion of Phase I study in cancer patients.

XIX. References

1. Lynch, K., *Theoretical and practical difficulties of developing a tumouristatic drug in the treatment of cancer.* Int. J. Pharm. Med., 1999. 13: 127.
2. Gore, M., A'Hern, R., Stankiewicz, M., and Slevin, M., *Tumour marker levels during marimastat therapy.* Lancet, 1996. 348(9022): 263.
3. Davies, B., Brown, P.D., East, N., Crimmin, M.J., and Balkwill, F.R., *A synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice bearing human ovarian carcinoma xenografts.* Cancer Res., 1993. 53: 2087.
4. Brown, P.D., *Matrix metalloproteinase inhibitors in the treatment of cancer.* Med. Oncol., 1997. 14(1): 1.
5. Yu, A.E., Hewitt, R.E., Connor, E.W., and Stetler-Stevenson, W.G., *Matrix metalloproteinases. Novel targets for directed cancer therapy.* Drugs Aging, 1997. 11(3): 229.
6. Powell, W.C. and Matrisian, L.M., *Complex roles of matrix metalloproteinases in tumor progression.* Curr. Top. Microbiol. Immunol., 1996. 213(Pt 1): 1.
7. Hagmann, W.K., Becker, J.W., and Lark, M.W., *Inhibition of matrix metalloproteinases*, in *Ann. Rep. in Med. Chem.*, J.A. Bristol, Editor. 1996, Academic Press: New York. p. 231.
8. Denis, L.J. and Verweij, J., *Matrix metalloproteinase inhibitors: present achievements and future prospects [In Process Citation].* Invest. New Drugs, 1997. 15(3): 175.

9. Kleiner, D.E. and Stetler-Stevenson, W.G., *Matrix metalloproteinases and metastasis.* Cancer Chemother. Pharmacol., 1999. 43(Sup. S): S42.

10. Gomez, D.E., Alonso, D.F., Yoshiji, H., and Thorgeirsson, U.P., *Tissue inhibitors of metalloproteinases: structure, regulation and biological functions.* Eur. J. Cell Biol., 1997. 74(2): 111.

11. Mitchell, P.G., Magna, H.A., Reeves, L.M., Lopresti-Morrow, L.L., Yocum, S.A., Rosner, P.J, Geoghegan, K.F., and Hambor, J.E., *Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage.* J. Clin. Invest., 1999. 97: 761.

12. Plantner, J, Smine, A., and Quinn, T., *Matrix metalloproteinases and metalloproteinases in human interphotoreceptor matrix and vitreous.* Curr. Eye Res., 1998. 17(2): 132.

13. Nagase, H., *Activation mechanisms of matrix metalloproteinases.* Biol. Chem., 1997. 378: 151.

14. Pei, D.Q., *Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP.* J. Biol. Chem., 1999. 274(13): 8925.

15. Strongin, A.Y., Collier, I., Bannikov, G., Marmer, B.L., Grant, G.A., and Goldberg, G.I., *Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease.* J. Biol. Chem., 1995. 270(10): 5331.

16. Powell, W.C., Knox, J.D., Narve, M., Grogan, T.M., Kittelson, J, Nagle, R.B., and Bowden, G.T., *Expression of the metalloproteinase matrilysin in DU-145 cells increases their invasive potetnial in severe combined immunodeficient mice.* Cancer Res., 1993. 53(2): 417.

17. Kawamata, H., Kameyama, S., Kawai, K., Tanaka, Y., Nan, L., Barch, D.H., Stetler-Stevenson, W.G., and Oyasu, R., *Marked acceleration o the metastatic phenotype of a rat bladder carcinoma cell line by the expression of human gelatinase A.* Int. J. Cancer, 1995. 63(4): 568.

18. Chambers, A.F. and Matrisian, L.M., *Changing views of the role of matrix metalloproteinase in metastasis.* J. Nat. Cancer Inst., 1997. 89(17): 1260.

19. Fowlkes, J.L., Enghild, J.J., Suzuki, K., and Nagase, H., *Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast-cultures.* J. Biol. Chem., 1994. 269(41): 25742.

20. Schnaper, H.W., Grant, D.S., and Stetler-Stevenson, W.G., *Type IV collagenase(s) and TIMPs modulate endothelial cell morphogenesis in vitro.* J. Cell Physiol., 1993. 156: 235.

21. Johnson, M.D., Kim, H.R., Chesler, L., Tsao-Wu, G., Bouck, N., and Polverini, P.J., *Inhibition of angiogenesis by tissue inhibitor of metalloproteinase.* J. Cell Physiol., 1994. 160: 194.

22. Hiraoka, N., Allen, E., Apel, I.J, Gyetko, M.R., and Weiss, S.J, *Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins.* Cell, 1998. 95(3): 365.

23. Stetler-Stevenson, W.G., *Matrix metalloproteinases in angiogenesis. A moving target for therapeutic intervention.* J. Clin. Invest., 1999. 103(9): 1237.

24. Anand-Apte, B., Pepper, M.S., Voest, E., Montesano, R., Olsen, B., Murphy, G., Apte, S.S., and Zetter, B., *Inhibition of angiogenesis by tissue inhibitor of metalloproteinase-3.* Invest. Ophthalmol. Vis. Sci., 1997. 38(5): 817.

25. Gress, T.M., Muller-Pillasch, F., Lerch, M.M., Friess, H., Buchler, M., and Alder, G., *Expression and in-situ localization of genes coding for extracellular matrix proteinase and extracellular matrix degrading proteases in pancreatic cancer.* Int. J. Cancer, 1995. 62: 407.

26. Davies, B., Miles, D.W., Happerfield, L.C., Naylor, M.S., Bobrow, L.G., Rubens, R.D., and Balkwill, F.R., *Activity of type IV collatgenases in benign and malignant breast disease.* Br. J. Cancer, 1993. 67: 1126.

27. Autio-Harmainen, H., Karttunen, T., Hurskainen, T., Hoyhtya, M., Kauppila, A., and Tryggvason, K., *Expression of 72 kilodalton type IV collagenase (gelatinase A) in benign and malignant ovarian tumors.* Lab. Invest., 1993. 69: 312.

28. Pyke, C., Ralfkiaer, E., Tryggvason, K., and Dano, K., *Messanger RNA for two type IVcollagenases is localized in stromal cells in human colon cancer.* Am. J. Pathol., 1993. 142: 359.

29. Kim, T.S. and Kim, Y.B., *Correlation between expression of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) and angiogenesis in colorectal adenocarcinoma.* J. Korean Med. Sci., 1999. 14: 263.

30. Polette, M., Clavel, C., Muller, D., Abecassis, J., Binninger, I., and Birembaut, P., *Detection of mRNAs encoding collagenase I and stromelysin 2 in carcinomas of the head and neck by in situ hybridization.* Invasion Metastasis, 1991. 11: 76.

31. Gray, S.T., Wilkins, R.J., and Yun, K., *Interstitial collagenase gene expression in oral squamous cell carcinoma.* Am. J. Pathol., 1992. 141: 301.

32. Basset, P., Okada, A., Chenard, M., Kannan, R., Stoll, I., Anglard, P., Bellocq, J., and Rio, M., *Matrix metalloproteinases as stromal effectors of human carcinoma progression: Therapeutic implications.* Matrix Biology, 1997. 15: 535.

33. Uria, JA., Stahle-Backdahl, M., Seiki, M., Fueyo, A., and Lopez-Otin, C., *Regulation of Collagenase-3 expression in human breast carcinomas is mediated by stromal-epithelial cell interactions.* Cancer Res., 1997. 57: 4882.

34. Shibata, K., Kikkawa, F., Nawa, A., Suganuma, N., and Hamaguchi, M., *Fibronectin secretion from human peritoneal tissue induces Mr 92,000 type IV collagenase expression and invasion in ovarian cancer cell lines.* Cancer Res., 1997. 57: 5416.

35. Hamasaki, T., Hattori, T., Kimura, G., and Nakazawa, N., *Tumor progression and expression of matrix metalloproteinase-2 (MMP-2) mRNA by human urinary bladder cancer cells.* Urol. Res., 1998. 26(6): 371.

36. Kuniyasu, H., Ellis, L.M., Evans, D.B., Abbruzzese, J.L., Fenoglio, C.J., Bucana, C.D., Cleary, K.R., Tahara, E., and Fidler, I.J., *Relative expression of E-cadherin and type IV collagenase genes predicts disease outcome in patients with resectable pancreatic carcinoma.* Clin. Cancer Res., 1999. 5(1): 25.

37. D'Errico, A., Garbisa, S., Liotta, L.A., Castronovo, V., Stetler-Stevenson, W.G., and Grigioni, W.F., *Augmentation of type IV collagenase, laminin receptor and Ki67 proliferation antigen associated with human colon, gastric and breast carcinoma progressio.* Mod. Pathol., 1991. 4: 239.

38. Murray, G.I., Duncan, M.E., O'Neil, P., Melvin, W.T., and Forthergill, J.E., *Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer.* Nature Med., 1996. 2: 461.

39. Engel, G., Heselmeyer, K., Auer, G., Backdahl, M., Eriksson, E., and Linder, S., *Correlation between stromelysin-3 level and outcome of human breast cancer.* Int. J. Cancer, 1994. 58: 830.

40. Zucker, S., Lysik, R.M., DiMassimo, B.I., Zarrabi, H.M., Moll, U.M., Grimson, R., Tickle, S.P., and Docherty, A.J., *Plasma assay of gelatinase B:tissue inhibitor of metalloproteinase complexes in cancer.* Cancer, 1995. 76: 700.

41. Moses, M.A., Wiederschain, D., Loughlin, K.R., Zurakowski, D., Lamb, C.C., and Freeman, M.R., *Increased Incidence of Matrix Metalloproteinases in Urine of Cancer Patients.* Cancer Res., 1998. 58: 1395.

42. Cockett, M.I., Murphy, G., Birch, M.L., O'Connell, J.P., Crabbe, T., Millican, A.T., Hart, I.R., and Docherty, A.J.P., *Matrix metalloproteinases and metastatic cancer.* Biochem. Soc. Symp., 1998. 63: 295.

43. Emmert-Buck, M.R., Emonard, H.P., Corcoran, M.L., Krutzsch, H.C., Foidart, J.M., and Stetler-Stevenson, W.G., *Cell surface binding of TIMP-2 and pro-MMP2/TIMP-2 complex.* FEBS Lett., 1995. 364: 28.

44. Brooks, P.C., Stromblad, S., Sanders, L.C., von Schalscha, T.L., Aimes, R.T., Stetler-Stevenson, W.G., Quigley, J.P., and Cheresh, D.A., *Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin αvβ3.* Cell, 1996. 85: 683.

45. Itoh, T., Tanioka, M., Yoshida, H., Yoshioka, T., Nishimoto, H., and Itohara, S., *Reduced Angiogenesis and Tumor Progression in Gelatinase A-deficient Mice.* Cancer Res., 1998. 58: 1048.

46. Bianco, F.J., Gervasi, D.C., Tiguert, R., Grignon, D.J, Pontes, J.E., Crissman, J.D., Fridman, R., and Wood, D.P., *Matrix metalloproteinase-9 expression in bladder washes from bladder cancer patients predicts pathological stage and grade.* Clin. Canc. Res., 1998. 4(12): 3011.

47. Ellerbroek, S.M., Hudson, L.G., and Stack, M.S., *Proteinase requirements of epidermal growth factor induced ovarian cancer cell invasion.* Int. J Cancer, 1998. 78(3): 331.

48. Sehgal, G., Hua, J., Bernhard, E.J., Sehgal, I., Thompson, T.C., and Muschel, R.J., *Requirement for matrix metalloproteinase-9 (gelatinase-B) expression in metastasis by murine prostate carcinoma.* Am. J Path., 1998. 152(2): 591.

49. Itoh, T., Tanioka, M., Matsuda, H., Nishimoto, H., Yoshioka, T., Suzuki, R., and Uehira, M., *Experimental metastasis is suppressed in MMP-9-deficient mice.* Clin. Exper. Metastasis, 1999. 17: 177.

50. Bergers, G., Coussens, L., Werb, Z., and Hanahan, D. *Tumor angiogenesis: mechanisms and therapeutic trials in transgenic mice.* presented at the *AACR Special Conference, Cancer Biology and the Mutant Mouse.* 1999. Keystone, Colorado.

51. Yu, Q. and Stamenkovic, I., *Localization of matrix metalloproteinase-9 to the cell surface provides a mechanism for CD44-mediated tumor invasion.* Genes Dev., 1999. 13(1): 35.

52. Yu, Q. and Stamenkovic, I., *Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis.* Genes Dev., 2000. 14(2): 163.

53. Ries, C., Loher, F., Zang, C.B., Ismair, M.G., and Petrides, P.E., *Matrix metalloproteinase production by bone marrow mononuclear cells from normal individuals and patients with acute and chronic myeloid leukemia or myelodysplastic syndromes.* Clin. Cancer Res., 1999. 5(5): 1115.

54. Janowska-Wieczorek, A., Marquez, L.A., Matsuzaki, A., Hashmi, H.R., Larratt, L.M., Boshkov, L.M., Turner, A.R., Zhang, M.C., and Edwards, D.R., *Expression of matrix metalloproteinases (MMP-2 and -9) and tissue inhibitors of metalloproteinases (TIMP-1 and -2) in acute myelogenous leukaemia blasts. Comparison with normal bone marrow cells.* Br. J Haematol., 1999. 105(2): 402.

55. Kuittinen, O., Savolainen, E.R., Koistinen, P., and Turpeenniemi-Hujanen, T., *Gelatinase-A and -B (MMP-2, MMP-9) in leukaemia MMP-2 may indicate a good prognosis in AML* Anticancer Res., 1999. 19(5C): 4395.

56. Brown, P.D. and Whittaker, M., *Matrix metalloproteinase inhibitors*, in *Antiangiogenic Agents in Cancer Therapy*, B.A. Teicher, Editor. 1999, Humana Press, Inc.: Totowa, NJ07512. p. 205.

57. Michaelides, M.R. and Curtin, M.L., *Recent advances in matrix metalloproteinase inhibitors research.* Curr. Pharm. Design, 1999. 5: 787.

58. Black, R.A., Rauch, C.T., Kozlosky, C.J, Peschon, J.J, Slack, J.L., Wolfson, M.F., Castner, B.J, Stocking, K.L., Reddy, P., Srinivasan, S., Nelson, N., Boiani, N., Schooley, K.A., Gerhart, M., Davis, R., Fitzner, J.N., Johnson, R.S., Paxton, R.J, March, C.J, and Cerretti, D.P., *A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells.* Nature, 1997. 385: 729.

59. Nemunaitis, J., Poole, C., Primrose, J., Rosemurgy, A., Malfetano, J., Brown, P., Berrington, A., Cornish, A., Lynch, K., Rasmussen, H., Kerr, D., Cox, D., and Millar, A., *Combined Analysis of Studies of the Effects of the Matrix Metalloproteinase Inhibitor Marimastat on Serum Tumor Markers in Advanced Cancer: Selection of a Biologically Active and Tolerable Dose for Longer-term Studies.* Clin. Cancer Res., 1998. 4: 1101.

60. Wojtowicz-Praga, S., Torri, J., Johnson, M., Steen, V., Marshall, J., Ness, E., Dickson, R., Sale, M., Rasmussen, H.S., Chiodo, T.A., and Hawkins, M.J, *Phase-I trial of marimastat, a novel matrix metalloproteinase inhibitor, administered orally to patients with advanced lung cancer.* J. Clin. Oncol., 1998. 16(6): 2150.

61. Shalinsky, D.R., Brekken, J., Zou, H., McDoermott, C.D., Forsyth, P., Edwards, D., Margosiak, S., Bender, S., Truitt, G., Wood, A., Varki, N.M., and Appelt, K., *Broad antitumor and antiangiogenic activities of AG3340, a potent and selective MMP inhibitor undergoing advanced oncology clinical trials.* Ann. N.Y. Acad. Sci., 1999. 878: 236.

62. Hande, K., Wilding, G., Ripple, G., Fry, J., Arzoomanian, R., Dixon, M., Yuen, G., and Collier, M. *A phase I study of AG3340, a matrix metalloprotease (MMP) inhibitor, in patients having advanced cancer.* presented at the *10th NCI-EORTC Symposium on New Drugs in Cancer Therapy*. 1998. Amsterdam.

63. Wilding, G., Small, E., Ripple, G., Keller, M., Tuen, G., and Collier, M. *Phase I study of AG3340, a matrix metalloprotease inhibitor, in combination with mitoxantrone/prednisone in patients having advanced prostate cancer.* presented at the *10th NCI-EORTC Symposium on New Drugs in Cancer Therapy*. 1998. Amsterdam.

64. Peschon, J.J, Slack, J.L., Reddy, P., Stocking, K.L., Sunnarborg, S.W., Lee, D.C., Russell, W.E., Castner, B.J., Johnson, R.S., Fitzner, J.N., Boyce, R.W., Nelson, N., Kozlosky, C.J., Wolfson, M.F., Rauch, C.T., Cerretti, D.P., Paxton, R.J., March, C.J., and Black, R.A., *An essential role for ectodomain shedding in mammalian development.* Science, 1998. 282(5392): 1281.

65. Glaser, K.B., Pease, L., Li, J.L., and Morgan, D.W., *Enhancement of the surface expression of tumor necrosis factor alpha (TNF-alpha) but not the p55 TNF-alpha receptor in the THP-1 monocytic cell line by matrix metalloprotease inhibitors.* Biochem. Pharmacol., 1999. 57(3): 291.

66. Rizvi, N.A., Humphery, J.S., Ness, E.A., Sonnichsen, D., Daly, D.J., Cooper, M., Conway, D., Marshall, J., and Hurwitz, H. *A Phase I, safety and pharmacokinetic trial of BMS-275291, a matrix metalloproteinase inhibitor (MMPI), in patients with advanced or metastatic cancer.* presented at the *AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Discovery, Development, and Clinical Validation*. 1999. Washington, D.C.

67. "*British Biotech plc Announces Results of Marimastat Study 193 in Advanced Pancreatic Cancer.*" Business Wire, January 25, 2000.

68. "*Marimastat encouraging in gastric cancer.*" Scrip/ PJB News, August 25, 1999.

69. Shalinsky, D.R., Brekken, J., Zou, H., Kolis, S., Wood, A., Webber, S., and Appelt, K., *Antitumor efficacy of AG3340 associated with maintenance of minimum effective plasma concentrations and not total daily dose, exposure or peak plasma concentrations.* Invest. New Drugs, 1998. 16(4): 303.

70. Tomayko, D.P. and Reynolds, C.P., *Determination of subcutaneous tumor size in athymic nude mice.* Cancer Chemother. Pharmacol., 1989. 24: 148.

71. Steel, G.G., Courtaney, V.D., and Peckman, M.J., *The response to chemotherapy of a variety of tumor xenographs.* Br. J. Cancer, 1983. 47: 1.

72. Houchens, D.P., Ovejera, A.A., and Barker, A.D., *Use of athymic nude mice in cancer research*, , D.P.H.a.A.A. Ovejera, Editor. 1978, Gustav Fisher: New York. p. 267.

73. Duivenvoorden, W.C., Hirte, H.W., and Singh, G., *Use of tetracycline as an inhibitor of matrix metalloproteinase activity secreted by human bone-metastasizing cancer cells.* Invasion Metastasis, 1997. 17(6): 312.

74. Kubota, S., Mitsudomi, T., and Yamada, Y., *Invasive human fibrosarcoma DNA mediated induction of a 92 kDa gelatinase/type IV collagenase leads to an invasive phenotype.* Biochem. Biophys. Res. Commun., 1991. 181(3): 1539.

75. Berrozpe, G., Schaeffer, J., Peinado, M.A., Real, F.X., and Perucho, M., *Comparative analysis of mutations in the p53 and K-ras genes in pancreatic cancer.* Int. J Cancer, 1994. 58(2): 185.

76. Morgan, D.W., *Efficacy evaluation of Abbott Laboratories' agents in the MetaMouse orthotopic model of human colon cancer Co-3.* AntiCancer, Inc. 2000, R&D/ 00/ 128.

77. Shimosato, Y., Kameya, T., Nagai, K., Hirohashi, S., Koide, T., Hayashi, H., and Nomura, T., *Transplantation of human tumors in nude mice.* J Natl Cancer Inst, 1976. 56(6): 1251.

78. An, Z., Wang, X., Willmott, N., Chander, S.K., Tickle, S., Docherty, A.J.P., Mountain, A., Millican, A.T., Morphy, R., Porter, J.R., Ola Epemolu, R., Kubota, T., Moossa, A.R., and Hoffman, R.M., *Conversion of highly malignant colon cancer from an aggressive to a controlled disease by oral administration of a metalloproteinase inhibitor.* Clin. Exp. Metastasis, 1997. 15: 184.

79. Kato, R. and Yamazoe, Y., *Sex-specific cytochrome P450 as a cause of sex- and species-related differences in drug toxicity.* Toxicol. Lett., 1992. 64/65: 661.

80. Tanaka, E., *Gender-related differences in pharmacokinetics and their clinical significance.* J. Clin. Pharm. Ther., 1999. 24: 339.

81. Wilson, K., *Sex-related differences in drug disposition in man.* Clin. Pharmacokin., 1984. 9: 189.

82. Boobis, A.R., Sesardic, D., Murray, B.P., Edwards, R.J., Singleton, A.M., Rich, K.J., Murray, S., de la Torre, R., Segura, J., and Pelkonen, O., *Species variation in the response of the cytochrome P-450-dependent monooxygenase system to inducers and inhibitors.* Xenobio., 1990. 20(11): 1139.

83. Diehl, M.S., *Bacterial reverse mutation assay of Abbott-291518 (Ames test).* Abbott Laboratories. 1999, Study No. Tx99-071.

84. Diehl, M.S., *Bacterial reverse mutation assay of Abbott-316023 (mini-screen Ames test).* Abbott Laboratories. 1999, Study Number TX99-151.

85. Synder, R.D., *In vitro micronucleus assay of Abbott metrix metalloproteinase inhibitors.* Abbott Laboratories. 1999,

86. Diehl, M.S., *In vitro micronucleus assay of A-316023.* Abbott Laboratories. 1999,

87. Glaser, K.B., *Cerep: study of A-291518.0 in various receptor binding assays.* Cerep. 2000, R&D/ 00/ 127.

88. Morgan, D.W., *A-291518.0 general CNS pharmacology profile in mice and rats after oral administration.* Porsolt & Partners Pharmacology. 2000, R&D/ 00/ 129.

89. Majors, K.R., Morgan, S.J., and Hanrahan, R.F., *Two-week oral toxicity study of Abbott-284770 in rats (with a two-week recovery period).* Abbott Laboratories. 1999, TA99-076.

90. Majors, K.R., Morgan, S.J., and Hanrahan, R.F., *Four-week oral toxicitiy study of Abbott-284770 in rats (with a two-week recovery period).* Abbott Laboratories. 1999, TA99-134.

91. Majors, K.R., Morgan, S.J., and Hanrahan, R.F., *Two-week oral toxicity study of Abbott-284770 in Cynomolgus monkeys (with a four-week recovery period).* Abbott Laboratories. 1999, TC99-075.

92. Majors, K.R., Morgan, S.J., and Hanrahan, R.F., *Four-week oral toxicity study of Abbott-284770 in Cynomolgus monkeys (with a four-week recovery period).* Abbott Laboratories. 1999, TC99-133.

93. Morgan, S.J and Brachen, W., *Four-week (with two-week interim) oral toxicity study in rats.* Abbott Laboratories. 1999, VA99-1284.

94. Gum, R.J, Heindel, M.A., Gagne, G.D., and Ulrich, R.G., *ABT-770, A-291518, A-316023: analysis of these MMP inhibitors and metobolites of all three compounds on inducing phopholipidosis in primary cultured rat and human hepatocytes.* Abbott Laboratories. 1999, R&D/ 99/ 217.

95. Cramer, C.T.a.U., R.G., *Cytotoxicity and lamellar body induction potential of a racemic benzamide antiarrhythmic compound and enantiomers in cultured rat hepatocytes.* Toxic. In Vitro, 1994. 8(5): 1083.

96. Ulrich, R.G., Kilgore, K.S., Sun, E.L., Cramer, C.T., and Ginsberg, L.C., *An in vitro fluorescence assay for the detection of drug-induced cytoplasmic lamellar bodies.* Toxicol. Meth., 1991. 1(2): 89.

97. Vu, T.H., Shipley, J.M., Bergers, G., Berger, J.E., Helms, J.A., Hanahan, D., Shapiro, S.D., Senior, R.M., and Werb, Z., *MMP-9 /gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes.* Cell, 1998. 93(3): 411.

98. Morgan, S.J., *Two-week continuous subcutaneous infusion to evaluate joint toxicity of marimastat in female rats.* Abbott Laboratories. 1999, VA99-937.

99. Larrey, D., Tinel, M., and Pessayre, D., *Formation of inactive cytochrome P-450 Fe(II)-metobolite complexes with several erythromycin derivatives but not with josamycin and midecamycin in rats.* Biochem. Pharmacol., 1993. 32: 1487.

100. Amacher, D.E., Schomaker, S.J, and Burkhardt, J.E., *The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies.* Food Chem. Toxicol., 1998. 36: 831.

101. Bracken, W.M. and Fickbohm, B.L., *Two-week oral toxicity and pharmacokinetic study of Abbott-316023 and Abbott-291518 administered to monkeys.* Abbott Laboratories. 1999, VC99-591.

102. Killar, L. *Inducible MMP-13 Transgenes.* presented at the *Matrix Metalloproteinase Gordon Research Conference.* 1999. Colby-Sawyer College, New London, New Hampshire.

103. Leff, R., *Clonical trials of a stromelysin inhibitor: osteoarthritis, matrix metalloproteinase inhibition, cartilage loss, surrogate markers, and clinical implications.* Ann. N.Y. Acad. Sci., 1999. 878: 201.

104. Bottomley, K., Borkakoti, N., Bradshaw, D., Brown, P., Broadhurst, M., Budd, J, Elliott, L., Eyers, P., Hallam, T., Handa, B., James, C.H.M., Lahm, H.-W., Lawton, G., Merritt, J, Nixon, J, Rothlisberger, U., Whittle, A., and Johnson, W., *Inhibition of bovine nasal cartilage degradation by selective matrix metalloproteinase inhibitors.* Biochem. J., 1997. 323: 483.

105. Wojtowicz-Praga, S.M., Dickson, R.B., and Hawkins, M.J., *Matrix metalloproteinase inhibitors.* Invest. New Drugs, 1997. 15(1): 61.

106. DiMartino, M., Wolff, C., High, W., Stroup, G., Hoffman, S., Laydon, J, Lee, J., Bertolini, D., Galloway, W., Crimmin, M., Davis, M., and Davies, S., *Anti-arthritic activity of hydroxamic acid-based pseudopeptide inhibitors of matrix metalloproteases and TNFα processing.* Inflamm. Res., 1997. 46: 211.

107. Felbor, U., *A second independent tyr-168cys mutation in the tissue inhibitor of metalloproteinase (TIMP-3) in Sorsby's fundus dystropy.* J Med. Gen., 1996. 33: 233.

108. Weber, B., *Mutations in the tissue inhibitor of metalloproteinase-3 (TIMP-3) in patients with Sorby's fundus dystrophy.* Nature Gen ., 1994. 8: 352.

109. Steen, B., *Matrix metalloproteinases and metalloproteinase inhibitors in choroidal neovascular membranes.* Invest. Ophthalmol. Vis. Sci., 1998. 39: 2194.

110. *"Angiogenesis; oral anti-angiogenesis drug AG3340 phase II study initiated."* Cancer Weekly Plus, October 6, 1998.

111. Niesman, M.R. *Ocular bioavailability and pharmacokinetics of AG3340, a potent inhibitor of metalloproteinases, following oral administration.* presented at the *Angiogenesis, Novel Therapeutic Development and Novel Antiangiogenic Therapy for Diseases of Ocular Neovascularization.* 1999. Boston, Massachusetts.

112. *InSite Vision: Q3 1997 Highlights.* Drugs Fut., 1998. 23(12): 1340.

113. Sorsa, T., Mantyla, P., Ronka, H., Kallio, P., Kallis, G.-B., Lundqvist, C., Kinane, D., Salo, T., Golub, L., Teronen, O., and Tikanoja, S., *Scientific basis of a matrix metalloproteinase-8 specific chair-side test for monitoring peridontal and peri-implant health and disease.* Ann. N.Y. Acad. Sci., 1999. 878: 130.

114. Golub, L., Ramamurthy, N., Llavaneras, A., Ryan, M., Lee, H., Liu, Y., Bain, S., and Sorsa, T., *A chemically modified nonantimicrobial tetracycline (CMT-8) inhibits gingival matrix metalloproteinases, peridontal breakdown, and extra-oral bone loss in ovariectomized rats.* Ann. N.Y. Acad. Sci., 1999. 878: 290.

115. Ashley, R. and Team, S.C.R., *Clinical trials of a matrix metalloproteinase inhibitor in human peridontal disease.* Ann. N.Y. Acad. Sci., 1999. 878: 335.

116. Celentano, D. and Frishman, W., *Matrix metalloproteinases and coronary artery disease: a novel therapeutic approach.* J Clin. Pharmacol., 1997. 37: 991.

117. Prescott, M., Sawyer, W., Linden-Reed, Jv., Jeune, M., Chou, M., Caplan, S., and Jeng, A., *Effect of matrix metalloproteinase inhibition on progression of atherosclerosis and aneurysm in LDL receptor-deficient mice overexpressing MMP-3, MMP-12, and MMP-13 and on restenosis in rats after balloon injury.* Ann. N.Y. Acad. Sci., 1999. 878: 179.

118. Loftus, I.M., Naylor, A.R., Goodall, S., Crowther, M., Jones, L., Bell, P.R.F., Thompson, M.M., and Thompson, M., *Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption.* Stroke, 2000. 31(1): 40.

119. Thompson, R. and Baxter, B., *MMP inhibition in abdominal aortic aneurysms: rationale for a prospective randomized clinical trial.* Ann. N.Y. Acad. Sci., 1999. 878: 159.

120. Treharne, G., Boyle, J., Goodall, S., Loftus, I., Bell, P., and Thompson, M., *Marimastat inhibits elastin degradation and matrix metalloproteinase 2 activity in a model of aneurysm disease.* Br. J. Surg., 1999. 86(8): 1053.

121. Mao, D., Lee, J., vanVickle, S., and Thompson, R., *Expression of collagenase-3 (MMP-13) in human abdominal aortic aneurysms and vascular smooth muscle cells in culture.* Biochem. Biophys. Res. Comm., 1999. 261(3): 904.

122. Li, Y., Geldman, A., Sun, Y., and McTiernan, C., *Differential expression of tissue inhibitors of metalloproteinases in the failing human heart.* Circulation, 1998. 98(17): 1728.

123. Spinale, F., Coker, M., Krombach, S., Mukherjee, R., Hallak, H., Houck, W., Clair, M., Kribbs, S., Johnson, L., Peterson, J., and Zile, M., *Matrix metalloproteinase inhibition during the development of congestive heart failure: effects on left ventricular dimensions and function.* Circ. Res., 1999. 85(4): 364.

124. Keiseier, B., Seifert, T., Giovannoni, G., and Hartung, H.-P., *Matrix metalloproteinases in inflammatory demyelination: Targets for treatment.* Neurology, 1999. 53: 20.

125. Steinmann-Niggli, K., Ziswiler, R., Kung, M., and Marti, H., *Inhibition of matrix metalloproteinases attenuates anti-Thy1.1 nephritis.* J. Am. Soc. Nephrol., 1998. 9(3): 397.

126. Wike-Hooley, J.L., van den Berg, A.P., van der Zee, J., and Reinhold, H.S., *Human tumour pH and its variation.* Eur. J. Cancer Clin. Oncol., 1985. 21(7): 785.

127. Millar, A.W., Brown, P.D., Moore, J, Galloway, W.A., Cornish, A.G., Lenehan, T.J, and Lynch, K.P., *Results of single and repeat dose studies of the oral matrix metalloproteinase inhibitor marimastat in healthy male volunteers.* Br. J Clin. Pharmacol., 1998. 45: 21.

128. Steward, W.P., *Marimastat (BB2516). Current status of development.* Cancer Chemother. Pharmacol., 1999. 43(Sup. S): S56.

129. Rosemugy, A., Harris, J, Langleben, A., Casper, E., and Allen, R., *Marimimastat, a novel matrix metalloproteinase inhibitor in patients with advanced carcinoma of the pancrease.* Proc. Am. Soc. Clin. Oncol., 1996. 15: 207a.

130. Zaknoen, S., Wolff, R., Cox, J., Marshall, J, Bordelon, T., Drumheller, A., and Rasmussen, H., *Marimastat in advanced progressive colorectoal cancer — a dose finding study.* Proc. Am. Soc. Clin. Oncol., 1997. 16: 273a.

131. Boasberg, P., Harbaugh, Eisnberg, M., Langleben, A., Ahmann, F., Roth, B., Berkheimer, M., and Rasmussen, H., *Marimastat in patients with hormone refractory prostate cancer: a dose finding study.* Proc. Am. Sci. Clin. Oncol., 1997. 16: 316a.

132. Malfetano, J., Teng, N., Barter, J, Lopez, A., Moore, D., Boasberg, P., Schink, J, Drumheller, A., and Rasmussen, H., *Marimastat in patients with advanced cancer of the ovary — a dose finding study.* Proc. Am. Soc. Clin. Oncol., 1997. 16: 373a.

133. Primrose, J.N., Bleiberg, H., Daniel, F., Van Belle, S., Mansi, J.L., Seymour, M., Johnson, P.W., Neoptolemos, J.P., Baillet, M., Barker, K., Berrington, A., Brown, P.D., Millar, A.W., and Lynch, K.P., *Marimastat in recurrent colorectal cancer. Exploratory evaluation of biological activity by measurement of carcinoembryonic antigen.* Br. J. Cancer, 1999. 79(3-4): 509.

134. Bramhall, S.R., *The matrix metalloproteinases and their inhibitors in pancreatic cancer. From molecular science to a clinical application.* Int. J. Pancreatol., 1997. 21(1): 1.

135. Rosemurgy, A., Harris, J., Langleben, A., Casper, E., Goode, S., and Rasmussen, H., *Marimastat in patients with advanced pancreatic cancer. A dose-finding study.* Am. J Clin. Oncol.-Cancer Clin.Trials, 1999. 22(3): 247.

136. Watson, S.A., Morris, T.M., Collins, H.M., Bawden, L.J., Hawkins, K., and Bone, E.A., *Inhibition of tumour growth by marimastat in a human xenograft model of gastric cancer. Relationship with levels of circulating CEA.* Br. J Cancer, 1999. 81(1): 19.

137. Brown, P. *The development and clinical application of broad-spectrum MMP inhibitors.* presented at the *Forbeck Symposium on Proteases as Cancer Therapeutic Targets.* 2000. Captiva Island, FL.

138. Rosemurgy, A., Buckels, J., Charnley, R., Winston, R., Steward, W., Staddon, A., Curtis, L., Rugg, T., and Rasmussen, H. *A randomized study comparing marimastat to gemcitabine as first line therapy in patients with non-resectable pancreatic cancer.* presented at the *35th American Society of Clinical Oncology.* 1999. Atlanta, Georgia.

139. Welch, M., *British Biotech's marimastat shows gastric cancer benefit.* BioWorld Today, 1999. 10(164).

140. Tierney, G.M., Griffin, N.R., Stuart, R.C., Kasem, H., Lynch, K.P., Lury, J.T., Brown, P.D., Millar, A.W., Steele, R.J.C., and Parsons, S.L., *A pilot study of the safety and effects of the matrix metalloproteinase inhibitor marimastat in gastric cancer.* Eur. J Cancer, 1999. 35(4): 563.

141. Moran, N., *British Biotech enters $60M deal on MMPIs with Schering-Plough.* BioWorld Today, 1999. 10(172): September 8.

142. Anderson, I., Supko, J., Eder, J., Vasconcelles, M., Shapiro, G., Skarin, A., Shipp, M., Janicek, M., Lynch, C., Lopez, M., Bower, M., and Rasmussen, H. *Pilot pharmacokinetics study if marimastat (MAR) in combination with carboplatin (C) |pactitaxel (T) in patients with metastatic or locally advanced*

inoperable non-small cell lung cancer (NSCLC). presented at the *35th American Society of Clinical Oncology*. 1999. Atlanta, Georgia.

143. Gradishar, W., VonRoenn, J., Cobleigh, M., Sparano, J., DeMichele, A., Schuchter, L., Zaring, D., and Rasmussen, H. *A phase I study of marimastat (MAR) in combination with doxorubicin (A) and cyclophosphamide (C) in patients with metastatic breast cancer.* presented at the *35th American Society of Clinical Oncology*. 1999. Atlanta, Georgia.

144. D'Olimpio, J, Hande, K., Collier, M., Michelson, G., Paradisco, L., and Clendeninn, N. *Phase I study of the matrix metalloproteinase inhibitor AG3340 in combination with paclitaxel and carboplatin for the treatment of patients with advanced solid tumors.* presented at the *35th American Society of Clinical Oncology Meeting*. 1999. Atlanta, Georgia.

145. Collier, M. *The development and clinical application of selective MMPI's.* presented at the *Forbeck Symposium on Proteases as Cancer Therapeutic Targets.* 2000. Captiva Island, FL.

146. Deal, J.G., Bender, S.L., Chong, W.K.M., Duvadie, R.K., Caldwell, M., Li, L., McTigue, M.A., Wickersham, J.A., Appelt, K., Almassy, R.J., Shalinsky, D.R., Daniels, R.G., McDermott, C.R., Brekken, J., Margosiak, S.A., Kumpf, R.A., Abreo, M.A., Burke, B.J, Register, J.A., Dagostino, E.F., Vanderpool, D.L., and Santos, O. *Preparation of various P1'-heterocyclic succinamide inhibitors of matrix metalloproteinases (MMPs).* presented at the *American Chemical Society*. 1999. Anaheim, California.

147. Sternlicht, M.D., Lochter, A., Sympson, C.J, Huey, B., Rougier, J., Gray, J.W., Pinkel, D., Bissell, M.J., and Werb, Z., *The stromal proteinase MMP3/stromelysin-1 promotes mammary carinogenesis.* Cell, 1999. 98: 137.

148. Ferrante, K., Winograd, B., and Canetta, R., *Promising new developments in cancer chemotherapy.* Cancer Chemother. Pharmacol., 1999. 43(Sup. S): S61.

149. Rowinsky, E., Hammond, L., Aylesworth, C., Humphrey, R., Smetzer, L., Siu, L., Weiss, G., Smith, L., Thurman, A., Rodriguez, G., Sorensen, M., Von Hoff, D., and Eckhardt, S.G. *Prolonged administration of BAY 12-9566, an oral non-peptidic biphenyl matrix: A phase I and pharmacologic study.* presented at the *10th NCI-EORTC Symposium on New Drugs in Cancer Therapy*. 1998. Amsterdam.

150. Sorbera, L.A., Graul, A., Silvestre, J., and Castaner, J., *Bay-12-9566.* Drugs Fut., 1999. 24(1): 16.

151. Rowinsky, E.K., Humphrey, R., Hammond, L.A., Aylesworth, C., Smetzer, L., Hidalgo, M., Morrow, M., Smith, L., Garner, A., Sorensen, J.M., Von Hoff, D.D., and Eckhardt, S.G., *Phase I and Pharmacologic Study of the Specific Matrix Metalloproteinase Inhibitor BAY 12-9566 on a Protracted Oral Daily Dosing Schedule in Patients with Solid Malignancies.* J. Clin. Oncol., 2000. 18(1): 178.

152. Erlichman, C., Adjei, A., Alberts, S., Sloan, J., Goldberg, R., Pitot, H., and Rubin, J., *Phase I Study of BAY 12-9566 - A Matrix Metalloproteinase Inhibitor (MMPI).* Proc. Am. Soc. Clin. Oncol., 1998. 17: 217a.

153. Erlichman, C., Adjei, A., Alberts, S., Sloan, J., Goldberg, R., Pitot, H., and Rubin, J. *Phase I study of BAY 12-9566 - a matrix metalloproteinase inhibitor (MMPI).* presented at the *34th American Society of Clinical Oncology*. 1998. Los Angeles, California.

154. "*Bayer drug casts shadow over MMP (matrix metalloproteinase) inhibitors in cancer.*" SCRIP/ PJB News, September 29, 1999.

155. Humphrey, R. *The development and clinical application of selective non-hydroxymate MMPI's.* presented at the *Forbeck Symposium on Proteases as Cancer Therapeutic Targets.* 2000. Captiva Island, FL.

156. Chouinard, E., Goel, R., Hirte, H.W., Stewart, D., Hurak, S., Waterfield, B., Matthews, S., Elias, I., and Seymour, L. *A phase I interaction study between BAY 12-9566 and doxorubicin in cancer patients.* presented at the *90th American Association of Cancer Research.* 1999. Philadelphia, Pennsylvania.

157. Michael, M., Babic, B., Khokha, R., Tsao, M., Ho, J., Pintilie, M., Leco, K., Chamberlin, D., and Shepard, F.A., *Expression and Prognostic Significance of Metalloproteinases and Their Tissue Inhibitors in Patients With Small-Cell Lung Cancer.* J. Clin. Oncol., 1999. 17(6): 1802.

158. Levitt, N.C., Eskens, F., Propper, D.J, Harris, A.L., Denis, L., Ganesan, T.S., Mather, R.A., McKinley, L., Planting, A., Talbot, D.C., Van Beurden, V., Van der Burg, M., Wilner, S., and Verweij, J., *A Phase One Pharmacokinetic Study of CGS27023A, A Matrix Metalloproteinase Inhibitor.* Proc. Am. Soc. Clin. Oncol., 1998. 17: 213a.

159. "*http://www.pharma.novartis.com/.*"

160. Lovejoy, B., Welch, A.R., Carr, S., Luong, C., Broka, C., Hendricks, R.T., Campbell, J.A., Walker, K.A.M., Martin, R., Van Wart, H., and Browner, M.F., *Crystal structures of MMP-1 and -13 reveal the structural basis for selectivity of collagenase inhibitors.* Nat. Struct. Biol., 1999. 6(3): 217.

161. Brewster, M., Lewis, E.J., Wilson, K.L., Greenham, A.K., and Bottomley, K.M.K., *Ro 32-3555, an orally active collagenase selective inhibitor, prevents structural damage in the STR/ORT mouse model of osteoarthritis.* Arthritis & Rheumatism, 1998. 41(9): 1639.

162. Wood, N.D., Aitken, M., Durston, S., Harris, S., McClelland, G.R., and Sharp, S., *Cartilage protection agent (CPA) Ro 32-3555, a new matrix metalloproteinase inhbitor for the treatment of rheumatoid arthritis.* Agents Actions Suppl., 1998. 49: 49.

163. Bradshaw, D. *Selective Metalloproteinase Inhibitors for the treatment of arthritis.* presented at the *Metalloproteases: Chemistry, Biology and Medicine.* 1999. Tamarron, Colorado.

164. Smith, G.N., Mickler, E., Hasty, K.A., and Brandt, K.D., *Specificity of Inhibition of Matrix Metalloproteinase Activity By Doxycycline.* Arthritis & Rheumatism, 1999. 42(6): 1140.

165. Lokeshaw, B.L., Houston-Clark, H.L., Selzer, M.G., Block, N.L., and Golub, L.M., *Potential Application of a Chemically Modified Non-Antimicrobial Tetracycline(CMT-3) Against Metastatic Prostate Cancer.* Adv. Dent. Res., 1998. 12(1): 1.

166. Patel, R.N., Attur, M.G., Dave, M.N., Patel, I.V., Stuchin, S.A., Abramson, S.B., and Amin, A.R., *A Novel Mechanism of Action of Chemically Modified Tetracyclines: Inhibition of COX-2-Mediated Prostaglandin E2 Production.* J. Immunol., 1990. 163: 3459.

167. Rudek, A., Dyer, V., Hamilton, J.M., Pluda, J.M., Reed, E., and Figg, W.D., *Preliminary Pharmacokinetics (PK) of Col-3, a Matrix Metalloproteinase (MMP) inhibitor.* Clin. Pharmacol. Ther., 1999. 65(2): 195.